European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

Glyphosate

Volume 3 – B.9 (AS)

Rapporteur Member State: Assessment Group on Glyphosate (AGG) consisting of FR, HU, NL and SE

Version History

When	What
2021/06	Initial RAR

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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B.9. ECOTOXICOLOGY DATA

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to Birds

Data point	CA 8.1.1.1/001
Report author	[redacted]
Report year	2003
Report title	MON 78623: An acute oral toxicity study with the Northern Bobwhite
Report No.	139-461
Document No.	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. OPPTS 850.2100
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	MON 78623
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7% glyphosate acid
Vehicle and/or positive control:	Deionised water Positive control: none
Test organism:	
Species:	Northern bobwhite, Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, 30 weeks
Sex:	Males and females
Weight:	176 - 248 g (at test initiation)
Source:	[redacted]
Food:	Game bird ration, *ad libitum* during acclimation and during the test, 18 h fasting prior to test start. Birds were given water soluble antibiotic in their drinking water for seven days after arrival in the laboratory.
Acclimation period:	Approx. 4 months
Environmental conditions:	
Temperature:	22.0 ± 0.2 °C
Relative humidity:	43% ± 8%
Photoperiod:	8 h light, 16 h dark

STUDY DESIGN AND METHODS

Replicates:	Ten quails (5 male, 5 female) were assessed per dose and control group. Each dosage group was assigned two pens. One pen contained five males and the other five females.
Treatments:	Nominal doses of 291, 484, 807, 1344 and 2241 mg glyphosate acid equivalent/kg bw by oral gavage. The control group was given diluent only.
Observations:	After test initiation, birds were observed twice daily for mortality, clinical signs of toxicity and abnormal behaviour. Body weights were measured at study initiation and after 3, 7 and 14 d. Average feed consumption was determined by pen for each group for day 0 – 3, 4 – 7 and 8 – 14, by measuring the weight change of the presented feed.
Analytical measurements:	Not reported.
Statistical analysis:	Since the mortality was <50%, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

RESULTS

There was no mortality observed.

Table B.9.1.1.1-1: Effects of glyphosate K-salt on body weight, food consumption of Northern bobwhite quail

Glyphosate K-salt [mg a.e./kg bw]	**Control**	**291**	**484**	**807**	**1344**	**2241**
Mortality						
Day 14	0	0	0	0	0	0
Clinical signs						
Ruffled appearance	0	0	2*	4	5	2
Lethargy	0	0	0	0	1	1
Mean body weight [g] (male/female)						
Day 0	224/197	221/208	222/207	219/206	219/221	225/212
Day 14	221/201	224/209	223/210	221/209	221/226	223/216
Feed consumption [g] (male/female)						
Day 0 – 3	31/15	28/21	27/26	18/23	21/15	17/23
Day 4 – 7	28/21	29/22	23/26	20/24	23/23	25/28
Day 8 - 14	25/16	24/17	19/20	21/20	20/18	17/18

* Not considered to be treatment related due to the timing and isolated nature of the signs noted.

One control male suffered a leg injury during body weight procedures and lost weight afterwards.

Numerous birds developed foot injuries during the study, which were proposed to be not treatment related. At 2241 mg a.e./kg bw one male received a foot injury. One male and one female in the 484 mg a.e./kg bw group got foot lesions with associated lameness and/or ruffled appearance. This was considered to be incidental to the treatment. At 807, 1344 and 2241 mg a.e./kg bw a number of birds showed a ruffled appearance. At 807 and 1344 mg a.e./kg groups all bird (except one male in 1344 mg a.e./kg group) had recovered by the morning of Day 11 of the test and were normal in appearance and behaviour for the remainder of the test. At the two highest test concentrations also lethargy was observed. No dose-response related increase of toxicity signs was noted.

When compared to the control group, no treatment related effects on body weight were noted except for the highest test concentration of 2241 mg a.e./kg bw. No treatment related effect on feed consumption was observed in female birds, while for males there seems to be a decreased intake throughout the study at doses from 484 mg a.e./kg bw. No statistical analysis was made for this parameter.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

CONCLUSIONS

Assessment and conclusion by applicant:

The acute LD_{50} for northern bobwhite exposed to glyphosate K-salt was determined to be > 2241 mg glyphosate acid equivalent/kg bw (nominal). The NOEC was determined to be 484 mg glyphosate acid equivalent/kg bw (nominal).

This study is considered valid and the acute oral LD_{50} for northern bobwhite exposed to glyphosate K-salt was determined to be > 2241 mg a.e./kg bw (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

It was noted by the former RMS, that according to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per bird is 1000 cm^2 for quail. In the present study the loading was 5 birds in 78 x 51 cm (796 cm^2 per bird), probably leading to unfavourable conditions to test animals. This may also partly explain the observed sublethal effects during the test.

The NOEL of 484 mg a.e./kg bw. proposed by the applicant was based on sublethal effects from 807 mg a.e./kg bw. Based on the observed decreased food consumption in male birds, a NOEL could be set to 291 mg a.e./kg bw. However, looking at the raw data it seems, that the body weight change of the concerned birds did not show the expected decrease. Instead they had a similar or better performance in the treatments compared with the control. The measurement of the leftover food in the feeders is not considered as reliable due to e.g. accidental spillage, behaviour of the test animals etc. Therefore, the old NOEC of 484 mg/kg bw can be retained. It is noted though, that this does not have an impact on the overall conclusion, since mortality is the crucial endpoint from this type of study.

Nevertheless, the validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. Except for the corrected NOEL based on male food consumption, the RMS agree to the results and conclusions of the APPL.

Data point	CA 8.1.1.1/002
Report author	[redacted]
Report year	1997
Report title	Glyphosate acid. Acute oral toxicity (LD_{50}) to Bobwhite quail
Report No.	[redacted] 400/963858
Document No.	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. Avian single dose LD_{50} test (1982)

Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6%
Vehicle and/or positive control:	Methylcellulose (1% w/v) Positive control: none
Test organism:	
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, approximately 4 - 6 month old on arrival
Weight:	175 - 213 g (15 days prior to test initiation)
Source:	Commercial supplier [redacted]
Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). Food was offered *ad libitum*, with the exception of an overnight starvation period of approximately 21 hours prior to dosing. Water was available at all times.
Acclimation period:	15 days
Environmental conditions:	
Temperature:	17 - 19°C
Relative humidity:	68 %
Photoperiod:	10 hours light / 14 hours darkness

STUDY DESIGN AND METHODS

Replicates:	5 males and 5 females per dosage
Treatments:	Young adult Bobwhite quails received a single dose of the test substance or vehicle by oral intubation using a disposable syringe and a Ch 10 Nelaton plastic catheter. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.e./kg bw (dosage concentrations: 5%, 10% and 20% w/v). A constant dose volume of 10 mL/kg bodyweight was used for all treatment groups. The control birds received an equivalent volume of methylcellulose only.
Observations:	During the test all mortalities, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of

the test. Feed consumption was determined by cage of each dosage group and the control group 15, 8, 7 and 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test.
Post mortem examination was carried out on all ten control birds and all ten birds from the highest dose group.

Analytical measurements: Not reported.

Statistical analysis: Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

RESULTS

There were no mortalities observed in any treatment.

Table B.9.1.1.1-2: Effects of glyphosate acid on body weight and food consumption of Bobwhite quail

Glyphosate acid [mg/kg bw]			**Control**	**500**	**1000**	**2000**
Average body weight per animal [g] (± SD)						
Body weight	Day -15	Male	192 ± 5.9	195 ± 5.9	192 ± 3.7	195 ± 4.9
		Female	191 ± 11.4	191 ± 15.6	191 ± 13.3	190 ± 8.9
	Day -7	Male	196 ± 5.7	196 ± 6.5	194 ± 4.1	198 ± 5.6
		Female	190 ± 10.2	190 ± 18.2	192 ± 7.8	189 ± 11.6
	Day 0	Male	194 ± 4.7	197 ± 6.9	193 ± 4.8	198 ± 5.9
		Female	190 ± 9.1	189 ± 17.1	192 ± 10.6	186 ± 10.5
	Day 7	Male	198 ± 2.5	199 ± 6.1	196 ± 4.3	198 ± 8.8
		Female	192 ± 13.0	192 ± 18.9	197 ± 13.3	191 ± 9.7
	Day 14	Male	200 ± 2.3	199 ± 4.9	196 ± 3.8	196 ± 7.0
		Female	192 ± 8.6	194 ± 17.0	198 ± 10.6	189 ± 9.5
Body weight change	Days 0-14	Male	6.0 ± 2.4	2.0 ± 2.0	3.0 ± 1.0	-2.0± 1.1
		Female	2.0 ± 0.5	5.0 ± 0.1	6.0 ± 0.0	3.0 ± 1.0
Mean food consumption per animal [g/bird/day]						
Food consumption	Day -15 to -8	Male	13	13	12	13
		Female	13	13	12	13
	Day -7 to -1	Male	13	13	12	13
		Female	13	13	13	13
	Day 1 to 7	Male	14	15	14	13
		Female	16	15	15	15
	Day 8 to 14	Male	14	14	14	13
		Female	15	13	14	14
Group mean	Day 1-14	Male	14	14.5	14	13
		Female	15.5	14	14.5	14.5

All control and test birds remained in good health following dosing, and no clinical signs of toxicity were observed. Body weight changes were similar in all groups and there was no evidence of any treatment-related effects. Group mean food consumption was similar in all groups and there was no evidence of any treatment-related effects. No abnormalities were detected in any birds during post mortem examination at termination of the study.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

CONCLUSIONS

Assessment and conclusion by applicant:
The acute oral LD_{50} for Bobwhite quail exposed to glyphosate acid was determined to be > 2000 mg a.e./kg bw. The NOEL in the study was determined to be 2000 mg a.e./kg bw.

This study is considered valid and the acute oral LD_{50} for Bobwhite quail exposed to glyphosate acid of > 2000 mg a.e./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:
According to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per bird is 1000 cm^2 for quail. In the present study the loading was 2-3 birds in 31 x 39 cm (400 – 600 cm^2), possibly leading to unfavourable conditions to test animals.

However, the validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. Overall, the RMS therefore agree to the results and conclusions of the APPL.

Data point	CA 8.1.1.1/003
Report author	[redacted]
Report year	1991
Report title	Glyphosate technical: Acute oral toxicity (LD_{50}) to the bobwhite quail
Report No.	[redacted] 48/91266
Document No.	-
Guidelines followed in study	FIFRA subdivision E, section 71-1
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate technical
Lot/Batch #:	206-JAK-119-1
Purity:	97.5%
Vehicle and/or positive control:	Methylcellulose (1% w/v) in distilled water Positive control: none
Test organism:	
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, approximately 16 weeks old on arrival
Weight:	180 g – 237 g at test start
Source:	Commercial supplier [redacted]

Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). This diet, though not analysed for contaminants, was known to contain no added antibiotic or other growth promoter. Food was offered *ad libitum*, with the exception of an overnight starvation period of approximately 17 hours on day -7 and prior to dosing. The starvation on day -7 was carried out in anticipation of dosing birds the following day. Due to an inadequate formulation of test material, however, dosing was delayed for a further week. Water was available at all times.
Acclimation period:	21 days
Environmental conditions:	
Temperature:	14 - 17 °C
Relative humidity:	82 %
Photoperiod:	10 hours light / 14 hours darkness

STUDY DESIGN AND METHODS

Replicates:	5 males and 5 females per treatment
Treatments:	Nominal dosages were 500, 1000 and 2000 mg/kg bw (dosage concentrations: 5%, 10% and 20% w/v). A constant dose volume of 10 mL/kg bodyweight was used for all treatment groups. Control birds received a corresponding volume of methylcellulose in distilled water only.
Observations:	During the test all mortalities, bird health and clinical signs of the birds were observed daily. Body weights were measured individually 21, 13, 6 and 0 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Feed consumption was determined by cage of each dosage group and the control group 21 to 14, 13 to 7, 6 to 1 day(s) prior to test start and on days 1 to 7 and 8 to 14 of the test. Post mortem examination was carried out on all ten birds from the highest dose group. For macroscopic post mortem examination the following tissues were examined: digestive tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat.
Analytical measurements:	Determination of the glyphosate concentration in each of the dose formulations, physical stability and chemical stability of the 1% methylcellulose formulations were performed by means of HPLC. The analytical method was assessed by the RMS as supportive but ‘fit for purpose’.
Statistical analysis:	Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEL was determined by visual interpretation of the mortality and observation data.

RESULTS

All birds remained in good health throughout the study and there were no mortalities observed.

Table B.9.1.1.1-3: Concentrations of glyphosate technical in dose formulations

Glyphosate technical [% w/v]	Analysed concentrations [% w/v]			Relative Mean Error [%]
	Analysis 1	Analysis 2	Mean	
0	ND	-	ND	-
5	4.80	5.37	5.09	+1.8
10	10.9	11.0	10.9	+9.0
20	20.7	19.3	20.0	+0.0

ND = Not detected (<0.015% w/v)

Mean measured results were within 9% of the nominal concentrations.

Table B.9.1.1.1-4: Effects of glyphosate technical on body weight and food consumption of bobwhite quail

Glyphosate technical [mg/kg bw]			Control	500	1000	2000
Average body weight per animal [g]						
Body weight	Day 0	male	207	211	206	207
		female	187	186	189	182
	Day 7	male	212	219	210	213
		female	190	191	193	188
	Day 14	male	213	222	213	216
		female	191	194	195	191
Mean food consumption per animal [g/bird/day]						
Food consumption	Day 0-7	male	19	20	18	18
		female	17	18	17	18
	Day 7-14	male	19	19	18	18
		female	19	18	18	18

Body weight changes were variable in all groups and there was no evidence of any treatment-related effect. With the exception of reduced consumption in group 3 over days -21 and -17, food consumption was similar in all groups with no evidence of any treatment-related effect.

No abnormalities were detected in any birds during post mortem examination at termination of the study. All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

CONCLUSIONS

Assessment and conclusion by applicant:

The acute oral LD_{50} of glyphosate technical to bobwhite quail was determined to be > 2000 mg a.s./kg bw. The NOEL in the study was 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for bobwhite quail exposed to glyphosate technical of > 2000 mg a.e./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:

According to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per

bird is 1000 cm^2 for quail. In the present study the loading was 5 birds in 38 x 75 cm (570 cm^2 per bird), possibly leading to unfavourable conditions to test animals.

However, the validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. Overall, the RMS therefore agree to the results and conclusions of the APPL.

Data point	CA 8.1.1.1/004
Report author	[redacted]
Report year	1999
Report title	Avian Single-Dose Acute Oral Toxicity Test in Japanese Quail with the chemical product Glifosate Técnico [redacted]
Report No.	D8.1–382/99
Document No.	-
Guidelines followed in study	Not stated
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No GLP stated in report
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	95% (nominal) 954.9 g/kg acid equivalent (measured)
Vehicle and/or positive control:	Gelatin capsules Positive control: none
Test organism:	
Species:	Japanese Quail (*Coturnix coturnix japonica*)
Age:	Young adults, at least 16 weeks old
Weight:	Males: 100 – 130 g at test start Females: 114 – 140 g at test start
Source:	Not stated
Food:	Commercial diet (GUABI ration) and water *ad libitum*.
Acclimation period:	At least 15 days
Environmental conditions:	
Temperature:	25 - 28°C
Relative humidity:	30 - 70%
Photoperiod:	10 hours light / 14 hours dark

STUDY DESIGN AND METHODS

Replicates:	5 males and 5 females per treatment

Treatments:	Single limit dose of 2000 mg a.s./kg bw of the test substance, enclosed in gelatin capsules. A control group received empty capsules by oral gavage.
Observations:	During the 15 days of the test, mortality, behaviour, clinical symptoms and anatomopathological alterations were observed daily. Birds were weighed at the beginning and at the end of test.
Analytical measurements:	The content of glyphosate in the technical material was measured by means of LC. The method was not evaluated in detail by the RMS, but is regarded as 'fit for purpose' since this is a vertebrate study.
Statistical analysis:	Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEL was determined by visual interpretation of the mortality and observation data (no statistical analysis).

RESULTS

There were no mortalities observed in any treatment.

Table B.9.1.1.1-5: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid [mg/kg bw]			**Control**	**2000**
Average body weight per animal [g] (± SD)				
Body weight	Day 0	male	109 ± 9.3	123 ± 5.3
		female	121 ± 5.8	122 ± 10.3
	Day 7	male	113 ± 11.1	119 ± 6.6
		female	122 ± 9.6	114 ± 9.9
	Day 14	male	119 ± 9.5	126 ± 6.9
		female	130 ± 9.6	124 ± 7.6
Body weight change	Days 0-14	male	10.2 ± 5.0	3.4 ± 5.5
		female	8.8 ± 7.4	1.8 ± 13.6
Mean food consumption per animal [g/bird/day]				
Food consumption	Day 0-7		111.3	99.4
	Day 7-14		77.2	99.6
Group mean	Day 0-14	mean	94.25	99.5

Body weight changes were variable within the groups and although the mean body weight increase were lower in the treated group, no treatment-related effects was proposed. Group mean food consumption was similar in all groups.

All control and test birds remained in good health following dosing, and no clinical signs of toxicity were observed. No abnormalities were detected in any birds during post mortem examination at termination of the study.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

CONCLUSIONS

Assessment and conclusion by applicant:

The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be >2000 mg a.e./kg bw. The NOEL in the study was determined to be 2000 mg a.e./kg bw.

This study is considered valid and the acute oral LD_{50} for Japanese quail exposed to glyphosate acid of >2000 mg a.e./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:

According to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per bird is 1000 cm^2 for quail. In the present study no information was given on the cage size.

However, the validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. Overall, the RMS therefore agree to the results and conclusions of the APPL.

Data point	CA 8.1.1.1/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Acute Oral Toxicity to Japanese Quail
Report No.	1413/4-1011
Document No.	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. Avian single dose LD_{50} test (1982)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	H95 D161A
Purity:	95.3%
Vehicle and/or positive control:	0.5% carboxymethyl cellulose (CMC) Positive control: none
Test organism:	
Species:	Japanese quail (*Coturnix coturnix japonica*)
Age:	Young adults, approx. 23 weeks old
Weight:	202 - 300 g (at test initiation)

Source: [redacted]
Food: Proprietary avian food, *ad libitum*
Acclimation period: 5 weeks prior to dosing

Environmental conditions:

Temperature: 15 – 20°C
Humidity: 40 – 78%
Photoperiod: 8 hours light / 16 hours dark

STUDY DESIGN AND METHODS

Replicates: Two groups of 5 males and 5 females per treatment and control.

Treatments: Single limit dose of 2000 mg a.e./kg bw (glyphosate acid dissolved in 0.5% carboxymethyl cellulose) by oral intubation. In addition, one control group was administered an equivalent volume of the vehicle (CMC) only as the test groups, at a dose rate of 2 mL/kg bw.

Observations: Birds were caged and observed continuously for signs of toxicity, abnormal behaviour and mortality for one hour after dosing, then at intervals throughout day 0 and twice daily thereafter. Food consumption was measured covering day 0-7, and day 7-14. Each animal was weighed at least on day 0, 3, 7 and 14. On day 14, all surviving animals were sacrificed, and a gross macroscopic examination was carried out. The necropsy comprised a general inspection of major visceral organs.

Analytical measurements: No analytical verification was performed.

Statistical analysis: Since the mortality was <50%, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

RESULTS

There was no mortality observed, except for one bird in treatment group found dead due to trauma of the reproductive tract.

Table B.9.1.1.1-6: Effects of glyphosate acid on body weight and food consumption of Japanese quail

Glyphosate acid [mg/kg bw]			**Control**	**2000**
Average body weight per animal [g] (± SD)				
Body weight	Day 0	Male	249 ± 27.1	228 ± 22.3
		Female	257 ± 15.3	260 ± 28.0
	Day 3	Male	270 ± 31.4	231 ± 22.2
		Female	268 ± 18.5	272 ± 36.1
	Day 7	Male	275 ± 31.8	239 ± 17.3
		Female	271 ± 18.5	271 ± 32.8
	Day 14	Male	276 ± 33.5	243 ± 18.5
		Female	276 ± 18.2	288 ± 28.7
Body weight change	Days 0-14	Male	26 ± 12	15 ± 5.7
		Female	19 ± 13.6	23 ± 3.0

Mean food consumption per animal [g/bird/day]				
Food consumption	Day 0-7	Male	64.5	39.9
		Female	56.1	60.9
	Day 7-14	Male	50.0	41.8
		Female	58.0	67.9
Group mean	Day 0-14	Mean	57.2	52.0

There were no adverse effects were observed on bodyweight or food intake, although the results were variable. No findings at necropsy were considered to be treatment-related.
All validity criteria according

CONCLUSIONS

Assessment and conclusion by applicant:
The acute oral LD_{50} for Japanese quail exposed to glyphosate acid was determined to be > 2000 mg a.e./kg bw. The NOEL in the study was determined to be 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for Japanese quail exposed to glyphosate acid of > 2000 mg a.e./kg bw can be used in risk assessment.

Assessment and conclusion by RMS:
According to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per bird is 1000 cm^2 for quail. In the test protocol of the present study, a minimum floor area of 250 cm^2 per bird was stipulated.

However, the validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. Overall, the RMS therefore agree to the results and conclusions of the APPL.

Data point	CA 8.1.1.1/006
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Acute Oral Toxicity to Mallard Duck
Report No.	1413/5-1011
Document No.	-
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1. Avian single dose LD_{50} test (1982)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate technical
Lot/Batch #:	H95 D161A
Purity:	95.3% w/w
Vehicle and/or positive control:	0.5% carboxymethyl cellulose (CMC) Positive control: none
Test organism:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	Young adults, approx. 23 weeks old
Sex:	Males and females
Weight:	903 - 1114g (at test initiation)
Source:	[redacted]
Food:	Proprietary avian food, *ad libitum*
Acclimation period:	5 weeks prior to dosing
Environmental conditions:	
Temperature:	15 – 22°C
Humidity:	42 – 74%
Photoperiod:	14 hours light / 10 hours dark

STUDY DESIGN AND METHODS

Replicates:	5 males and 5 females per treatment group (males and females separately).
Loading:	Approx. 4.5 m^2 for 5 birds
Treatments:	Single limit dose of 2000 mg a.s./kg bw (technical glyphosate dissolved in 0.5% carboxymethyl cellulose) by direct intubation. In addition, one control group comprising 5 males and 5 females was administered an equivalent volume of the vehicle (CMC) only, at a dose rate of 2 mL/kg bw.
Observations:	Birds were caged and observed for signs of toxicity, abnormal behaviour and mortality continuously for one hour after dosing, then at intervals throughout day 0 and twice daily thereafter. Food consumption was measured per time interval, covering day 0-7, and day 7-14. Each animal was weighed at least on day 0, 5, 11 and 14. On day 14, all surviving animals were sacrificed and a gross macroscopic examination was carried out. The necropsy comprised a general inspection of major visceral organs.
Analytical measurements:	Not reported.
Statistical analysis:	Since no mortality was reported, no statistical calculation of LD_{50} values was possible. The NOEL was determined by visual interpretation of the mortality and observation data.

RESULTS

No mortalities and no post-dosing signs of toxicity were observed in any treatment and all animals remained in good health throughout the study.

Table B.9.1.1.1-7: Effects of glyphosate technical on body weight and food consumption of Mallard duck

Glyphosate technical [mg a.s./kg bw]			Control	2000
Average body weight per animal [g] (± SD)				
Body weight	Day 0	Male	1011 ± 41.5	1012 ± 76.4
		Female	1072 ± 128.4	1018 ± 81.1
	Day 5	Male	1101 ± 33.5	1048 ± 49.6
		Female	1170 ± 160.0	1082 ± 60.8
	Day 11	Male	1096 ± 54.8	1052 ± 69.6
		Female	1191 ± 155.0	1175 ± 41.4
	Day 14	Male	1104 ± 51.8	1053 ± 65.9
		Female	1171 ± 122.6	1156 ± 66.5
Body weight change	Days 0-14	Male	93 ± 30.9	42 ± 12.2
		Female	99 ± 86.0	138 ± 110.8
Mean food consumption per animal [g/bird/day]				
Food consumption	Day 0-7	Male	79	80
		Female	131	121
	Day 7-14	Male	72	76
		Female	130	138

The body weight was not adversely affected by the treatment, although there was a large variation within the groups. There were equally no treatment-related effects on food consumption and no abnormalities were detected at necropsy of the animals 14 days after treatment.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

CONCLUSIONS

Assessment and conclusion by applicant:

The acute oral LD_{50} for Mallard duck exposed to glyphosate technical was determined to be >2000 mg a.s./kg bw. The NOEL was determined to be 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for Mallard duck exposed to glyphosate technical was determined to be > 2000 mg a.s./kg bw and can be used in risk assessment.

Assessment and conclusion by RMS:

Some deviations regarding test conditions were noted compared to OECD 223; the birds were not individually caged but kept in groups of five, photoperiod was 10 hours light instead of 8 hours. Further, a large variation in humidity was observed. These deviations are however not considered to invalidate the study.

The validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. The RMS therefore agree to the results and conclusions of the APPL.

Data point	CA 8.1.1.1/007
Report author	[redacted]
Report year	1992

Report title	Glyphosate technical: Acute oral toxicity (LD_{50}) to mallard duck
Report No.	[redacted] 49/91843
Document No.	AVS94-00229
Guidelines followed in study	FIFRA subdivision E, section 71-1
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviation compared with OECD 223 – none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate technical
Lot/Batch #:	206-JAK-119-1
Purity:	97.5%
Vehicle and/or positive control:	Methylcellulose (1% w/v) in distilled water Positive control: none
Test organism:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	Approximately 22 month old at test start
Weight:	970 g – 1250 g
Source:	Commercial supplier [redacted]
Food:	Standard HRC layer diet in pellet form obtained from Parker Brothers Ltd. (Lark Mills, Mildenhall, Suffolk, UK). Food was offered *ad libitum*, with the exception of an overnight starvation period of approximately 19 hours prior to dosing. Water was available at all times.
Acclimation period:	15 days
Environmental conditions:	
Temperature:	11 – 16 °C
Relative humidity:	92%
Photoperiod:	10 hours light / 14 hours darkness

STUDY DESIGN AND METHODS

Replicates:	5 males and 5 females per treatment group (males and females separately)
Loading:	5 animals per cage, 184 x 120 cm
Treatments:	Single dose of the test substance or vehicle by oral intubation using a disposable syringe and a Ch 10 Nelaton plastic catheter. The test consisted of three dosage groups and a control group. Nominal dosages used in the study were 500, 1000 and 2000 mg a.s./kg bw (dosage concentrations: 10%, 20% and 40% w/v glyphosate technical). A constant dose volume of 5 mL/kg bodyweight was used for all treatment

groups. The control birds received a corresponding volume of methylcellulose in distilled water only.

Observations: Birds were observed daily during the study and at frequent intervals during the post-treatment period. Mortalities, bird health and clinical signs were recorded at each observation. Individual body weights were measured individually 15 and 7 days prior to test start, at the initiation of the test (immediately prior to dosing) and on days 7, and 14 of the test. Group mean food consumption was determined over days 15 to 8 and 7 to 1 prior to test start and on days 1 to 7 and 8 to 14 days after treatment. Post mortem examination was carried out on any bird which died during the study an on twenty birds from the highest dose groups in which there were survivors.

Analytical measurements: Determination of the glyphosate concentration in each of the dose formulations, physical stability and chemical stability of the 1% methylcellulose formulations were performed. The analytical method was assessed by the RMS as Supportive but 'fit for purpose'.

Statistical analysis: Descriptive statistics.

RESULTS

On day 6 one male bird of one control group was found dead. This was possibly associated with the aggressive behaviour of one other male bird observed (as described below). There were no other mortalities in any treatment group.

Table B.9.1.1.1-8: Concentrations of glyphosate technical in dose formulations

Glyphosate technical [% w/v]	Analysed concentrations [% w/v]			Relative Mean Error [%]
	Analysis 1	Analysis 2	Mean	
0	ND	-	ND	-
10	11.0	9.16	10.1	+1.0
20	25.4	18.5	22.0	+10.0
40	39.1	37.5	38.3	-4.3

Mean results of the analytical measurements were within 10% of the nominal concentrations.

Table B.9.1.1.1-9: Effects of glyphosate technical on body weight and food consumption of mallard duck

Glyphosate technical [mg a.s./kg bw]			Control	500	1000	2000
Average body weight per animal [g]						
Body weight	Day 0	Male	1036	1033	1066	1038
		female	1034	1015	971	981
	Day 7	Male	1098	1103	1142	1119
		female	1090	1079	1010	1042
	Day 14	Male	1189	1129	1156	1132
		female	1092	1075	1012	1036
Mean food consumption per animal [g/bird/day]						
Food consumption	Day 1-7	Male	88	91	103	117
		female	103	100	89	97
	Day 8-14	Male	100	114	114	111
		female	91	80	86	89

Body weight changes were variable in all groups and there was no evidence of any treatment-related effect.

All birds remained in good health throughout the study and there were no clinical signs of toxicity. One male bird in one of the control groups became aggressive towards other birds in the group on day 7. This bird was removed from the pen and housed separately until the end of the study.

One male bird of the highest treatment group (glyphosate technical: 2000 mg a.s./L) was found to have a fluid-filled body cavity, and one lobe of the liver was bulbous and had a fibrous coating. This was not considered to be treatment-related. No other abnormalities were detected in any other bird examined.

No non-incidental death was observed in the control groups. In contrast to guideline OECD 223, the total number of control birds used in the test was ten instead of five. Therefore, although one bird died incidentally, all validity criteria according to OECD 223 were fulfilled.

CONCLUSIONS

Assessment and conclusion by applicant:
Under the conditions of this study the acute oral LD_{50} of glyphosate technical to mallard duck was found to be >2000 mg a.s./kg bw. The NOEL in the study was 2000 mg a.s./kg bw.

This study is considered valid and the acute oral LD_{50} for mallard duck exposed to glyphosate technical was determined to be >2000 mg a.e./kg bw and can be used in risk assessment.

Assessment and conclusion by RMS:
Some deviations regarding test conditions were noted compared to OECD 223; the birds were not individually caged but kept in groups of five, photoperiod was 10 hours light instead of 8 hours. These deviations are however not considered to invalidate the study.

The validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. The RMS therefore agree to the LD_{50} proposed by the APPL.

Due to the observed sublethal effects in one bird the highest treatment level, a possible dose-response effect cannot be excluded. Therefore, the NOEL should be set to 1000 mg a.s./kg bw.

Data point	CA 8.1.1.1/008
Report author	[redacted]
Report year	1983
Report title	Report of the acute oral toxicity (MLD) to pigeon with glyphosate (tech) of [redacted]
Report No.	[redacted] 95-00214
Document No.	-
Guidelines followed in study	No information mentioned in the Monograph 2001.
Deviations from current test guideline identified by the applicant:	-

See RMS analysis in RMS comment box	
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No (information from the reference list of the Monograph 2001)
Acceptability/Reliability	RMS: Not reliable

Short description of study design and observations:	Acute oral toxicity of glyphosate (tech) to pigeon.
Short description of results:	No information mentioned in the Monograph.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	No study report available and no information mentioned in the Monograph 2001.
Reasons why the study report is not available for submission:	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS:

The AGG requested this study from BVL and it has been checked for reliability. Agree with the conclusion from the previous evaluation that the study is not reliable. Information on identity of the test substance, source of test animals and on test conditions was scarce, and the number of replicates were too low to obtain reliable results. Some mortalities and sublethal effects (ataxia and loss of righting reflex) were observed, but at doses higher than those already tested in other available studies.

Data point	CA 8.1.1.1/009
Report author	[redacted]
Report year	1991
Report title	AMPA: An Acute Oral Toxicity Study with the Northern Bobwhite
Report No.	139-277
Document No.	-
Guidelines followed in study	FIFRA Guideline 71-1
Deviations from current test guideline identified by the applicant:	Deviation compared with OECD 223 – none
See RMS analysis in RMS comment box	
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	AMPA

Lot/Batch #:	PIT-9008-2407T
Purity:	97% (nominal), 87.8% (measured)
Vehicle and/or positive control:	Corn oil (diluent) Positive control: none
Test organism:	
Species:	Northern bobwhite quail (*Colinus virginianus*)
Age:	18 weeks old
Sex:	Males and females
Weight:	164 – 220 g (at test initiation)
Source:	[redacted]
Food:	Game bird ration, *ad libitum* during acclimation and during the test.
Acclimation period:	16 days
Environmental conditions:	
Temperature:	21 ± 1°C
Humidity:	41 ± 15%
Photoperiod:	8 hours light / 16 hours dark (approx.. 130 lux)

STUDY DESIGN AND METHODS

Replicates:	Five birds/pen and two pens/dose
Loading:	Cage size 78 x 51 cm for 5 birds
Treatments:	292, 486, 810, 1350 and 2250 mg/kg bw, dissolved in corn oil by oral gavage. The control group was dosed with the diluent only.
Observations:	After dosing, the birds were observed at least twice daily for 14 days for mortality, signs of toxicity, or abnormal behaviour. Body weights were measured individually at initiation of the test and by group on days 3, 7 and 14. Average estimated feed consumption was determined for each dosage group and the control for days 0-3, 4-7 and 8-14.
Analytical measurements:	No analytical verification was performed.
Statistical analysis:	Descriptive statistics

RESULTS

There were no mortalities at any of the dosages tested.

Table B.9.1.1.1-10: Cumulative mortality and clinical signs of toxicity observed in Northern bobwhite quail exposed AMPA

AMPA [mg/kg bw]	**Control**		**292**		**486**		**810**		**1350**		**2250**	
	M	F	M	F	M	F	M	F	M	F	M	F
Mean cumulative mortality on day 14 [%]	0	0	0	0	0	0	0	0	0	0	0	0
Appeared normal [a]	5	5	5	5	5	5	5	5	5	5	0	3
Reduced reaction [a]	-	-	-	-	-	-	-	-	-	-	4	2
Ruffled appearance [a]	-	-	--		-	-	-	-	-	-	4	2
Lower limb weakness [a]	-	-	-	-	-	-	-	-	-	-	2	-

[a] Clinical signs of toxicity were only noted on day 0 only
M = male, F = females

Table B.9.1.1.1-11: Effects of AMPA on body weight and food consumption of bobwhite quail

AMPA [mg/kg bw]			Control	292	486	810	1350	2250
Average body weight per animal [g]								
Body weight	Day 0	male	188	181	187	195	187	180
		female	181	184	181	173	182	188
	Day 7	male	197	188	194	204	191	187
		female	189	192	190	177	185	193
	Day 14	male	200	192	200	203	195	191
		female	192	197	196	183	190	201
Mean food consumption per animal [g/bird/day]								
Food consumption	Day 0-3	male	23	23	16	25	16	18
		female	18	16	17	16	16	19
	Day 4-7	male	25	26	19	23	23	20
		female	21	21	22	20	18	22
	Day 8-14	male	22	24	21	24	24	20
		female	21	19	24	22	18	19

Birds were normal in appearance and behaviour throughout the test period, although one male at 810 mg/kg bw was noted with foot lesions due to pen-wear on day 13 and 14.

At a dosage 2250 mg/kg bw, signs of toxicity were first noted approximately fifty-five minutes after dosing and persisted through the afternoon of day 0. By the morning of day 1, all birds were noted as normal in appearance and behaviour and remained so until study termination.

Signs of toxicity characteristic of intoxication with AMPA included lower limb weakness, a ruffled appearance, and reduced reaction to external stimuli (sound and movement). When compared to the controls, no notable effect on body weight or feed consumption was observed at any of the dosages tested.

All validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups.

CONCLUSIONS

Assessment and conclusion by applicant:
The acute oral LD_{50} for northern bobwhite quail exposed to AMPA as a single oral dosage was > 2250 mg/kg bw. The NOEL was 1350 mg/kg bw.

This study is considered valid and the acute oral LD_{50} for northern bobwhite quail exposed to AMPA as a single oral dosage was > 2250 mg/kg bw and can be used in risk assessment.

Assessment and conclusion by RMS:
According to OECD guideline this study is designed to have birds housed individually and housing conditions should be within optimal limits for the test species and minimum recommended space per bird is 1000 cm^2 for quail. In the test protocol of the present study, the cage floor area was 78 x 51 cm for 5 birds (796 cm^2 per bird).

However, the validity criteria according to OECD 223 were fulfilled, as no non-incidental death was observed in the control groups. Overall, the RMS therefore agree to the results and conclusions of the APPL.

B.9.1.1.2. Short-term dietary toxicity to birds

Short term avian dietary studies are not key data requirements according to EU Reg. 283/2013, but should be considered when available in order to support the assumption that the acute risk assessment is comparable or higher than the toxicity from dietary exposure. Summaries of the available studies are presented below.

Data point	CA 8.1.1.2/01
Report author	[redacted]
Report year	1973
Report title	Eight-day dietary LC_{50} – Bobwhite quail technical CP67573
Report No.	241-106
Document No.	-
Guidelines followed in study	Not mentioned
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	The following deviations from test guideline OECD 205 (1984) were reported: The acclimation period duration is not reported (min of 7 days is required) Mortality during 72h before exposure should be assessed in order to validate the batch of organisms (not reported) The spacing factor between concentrations should not exceed 2 (2.15 was used) Bodyweights not determined for Day 5, only day 0 and day 8 reported.
Previous evaluation	Yes, evaluated and accepted in Monograph 2001
GLP/Officially recognised testing facilities	No, not reported
Acceptability/Reliability	Not acceptable, did not fulfil validity criteria. See below.

MATERIAL AND METHODS

Test material:	Technical CP67573 (glyphosate)
Lot/Batch #:	LH 14, 583A
Purity:	98.5% glyphosate
Vehicle and/or positive control:	Corn oil included in the basal diet Positive control: Dieldrin
Test organism:	
Species:	Northern bobwhite, Bobwhite quail (*Colinus virginianus*)

RESULTS

No analytical verification of the treated diets were mentioned in the report. The concentrations and LC_{50} value given below are based on nominal doses.

There were no mortalities or overt signs of toxicity in the control or treatment group. The dietary LC_{50} and NOEC for northern bobwhite exposed to Technical CP67573 were determined to be > 4640 and 4640 ppm, respectively (nominal).

Validity criteria according to the current OECD guideline 205 (1984):
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%)
- The lower test item concentration resulted in no mortality and no observable toxic effects.

The following validity criterion was not met:

- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (no analytical results were reported).

Some data are missing in order to be able to conclude on the achievement of the validity criteria.

CONCLUSIONS

Assessment and conclusion by applicant:
The dietary LC_{50} and NOEC for northern bobwhite exposed to glyphosate (Technical CP67573) were determined to be > 4640 ppm (nominal), which is calculated to be equivalent to LD_{50} >1012.4 mg a.s./kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:
The study has not been evaluated in detail by the RMS. However, based the limitations pointed out by the GRG, especially those related to the validity criteria of OECD TG 205, the study should not be used further for the risk assessment.

Data point	CA 8.1.1.2/02
Report author	[redacted]
Report year	1997
Report title	Glyphosate acid: Dietary LC_{50} to the bobwhite quail
Report No.	[redacted] 395/963857
Document No.	-
Guidelines followed in study	EPA FIFRA 71-2 (1982) and OECD 205 (1984)
Deviations from current test guideline identified by the applicant:	According to applicant, none significant compared with OECD 205 (1984)
See RMS analysis in RMS comment box	
Previous evaluation	Submitted but not used in RAR 2015
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Acceptable. See RMS' comments below.

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6%
Vehicle and/or positive control:	Not required Positive control: none
Test organism:	
Species:	Northern bobwhite, Bobwhite quail (*Colinus virginianus*)
Age:	Ten days old at test start
Number of animals:	10 birds per pen; 2 pens in control group, 5 pens in treatment
Weight:	14.5 – 15.7 g (at test initiation)
Source:	[redacted]
Food:	Standard HRC chick diet *ad libitum* during acclimation and during the test.
Acclimation period:	3 days
Environmental conditions:	
Temperature:	25 - 27 °C
Relative humidity:	33%
Photoperiod:	10 hours light / 14 hours darkness

STUDY DESIGN AND METHODS

Replicates:	-
Treatments:	The test consisted of a geometric series of five test concentrations and two control groups. Nominal dietary concentrations used in this study were 325, 650, 1300, 2600 and 5200 mg/kg diet. Each group was fed the appropriate test or control diet for five days. Following the five day exposure period all groups were given untreated feed for three days.
Observations:	Birds were observed daily during the study and at frequent intervals during the treatment and post-treatment periods. Mortalities, bird health and clinical signs, including appearance of excreta, were recorded at each observation. Group mean body weights were recorded at days -3, 1 (immediately prior to introduction of test diets), 5 and 8. Group mean food consumption was determined over days -3 to -1, 1 to 5 (daily) and 6 to 8. Post mortem examination was carried out on all ten birds from the highest dose group and ten birds from the control groups. For macroscopic post mortem examination the following tissues were examined: gastrointestinal tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat. The birds were observed for any gross pathological changes.
Analytical measurements:	Yes, by means of HPLC. The analytical method was not evaluated in detail by the RMS, but is regarded as 'fit for purpose' since this is a vertebrate study.
Statistical analysis:	Not reported. No mortality occurred.

RESULTS

Clinical signs and mortalities: All birds remained in good health throughout the study and there were no mortalities in either the control or treatment groups. Excreta were normal in appearance in all groups throughout the study.

Body weight: Body weight changes were variable in all groups and there was no evidence of any treatment-related effect.

Feed consumption: Food consumption was similar in all groups and there was no evidence of any treatment-related effects.

Macroscopic post mortem examination: No abnormalities were detected in any birds during post mortem examination at termination of the study.

Analytical verifications of the treated diets were between 96.3 and 107.5 % of nominal. The concentrations and LC_{50} values given below are based on nominal doses.

Table B.9.1.1.2-1: Summary of effects of glyphosate acid on mortality of northern bobwhite (*Colinus virginianus*) following dietary oral exposure

Treatment (ppm)	8-day cumulative mortality [nb dead/nb exposed]	Mean body weight day 0 [g]	Mean body weight day 5 [g]	Mean food consumption over 5 days [g/bird/day]
Control (0)	0/10	15.1	22.9	4.7
Control (0)	0/10	15.4	22.4	5.2
Glyphosate (325)	0/10	15.0	22.0	5.0
Glyphosate (650)	0/10	14.9	22.2	5.3
Glyphosate (1300)	0/10	14.9	21.1	4.9
Glyphosate (2600)	0/10	15.7	22.6	5.5
Glyphosate (5200)	0/10	14.5	21.3	5.2

There were no mortalities or overt signs of toxicity in the control or treatment group. Since there was no mortality, it was not possible to perform the calculation of an exact LC_{50} value. No treatment related effect on mortality was observed. The dietary LC_{50} and NOEC for northern bobwhite exposed to glyphosate acid were determined to be > 5200 ppm (nominal).

Validity criteria according to the current OECD guideline 205 (1984):
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%)
- The lower test item concentration resulted in no mortality and no observable toxic effects.
- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (actual 96.3 and 107.5 % of nominal).

CONCLUSIONS

Assessment and conclusion by applicant:
The dietary LC_{50} and NOEC for northern bobwhite exposed to glyphosate acid were determined to be > 5200 ppm (nominal), which is calculated to be equivalent to LD_{50} >1510.6 mg a.e./kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:
The study was not evaluated in detail by the RMS, however, it seems to comply with the relevant validity criteria. There is no indication that dietary exposure to glyphosate is more severe than from the acute oral exposure. The study is considered as acceptable as the results are only used to confirm the low toxicity concluded from the available acute oral data.

Data point	CA 8.1.1.2/03
Report author	[redacted]
Report year	1989
Report title	Dietary toxicity study in Japanese quail with glyphosate technical
Report No.	1085
Document No.	-
Guidelines followed in study	OECD guideline 205
Deviations from current test guideline identified by the applicant:	The light should be between 12-16 hours (24 hours light used). Two control groups of 10 birds each are required (only 10 birds were tested).
See RMS analysis in RMS comment box	
Previous evaluation	Yes, evaluated and accepted Monograph 2001
GLP/Officially recognised testing facilities	Yes, conducted under GLP officially recognised testing facilities
Acceptability/Reliability	Not acceptable, did not fulfil validity criteria (see below).

MATERIAL AND METHODS

Test material:	Glyphosate Technical
Lot/Batch #:	38
Purity:	95%
Vehicle and/or positive control:	The basal diet Positive control: none
Test organism:	
Species:	Japanese quail (*Coturnix coturnix japonica*)

STUDY DESIGN AND METHODS

Treatments:	The test consisted of a limit test concentration for the test item and a control group. Twenty Japanese quail birds were randomly assigned to the control and the treated groups, after seven days of acclimation and acceptance of the batch (mortality within 3 days before test initiation was 0%). The nominal concentration of glyphosate, administered by food mixing, was 5000 ppm. Diets were presented to the birds at initiation of the test.
Observations:	Test birds were observed for sings of toxicity, twice on the first day of exposure and then daily for eight days. Mortality was

recorded through the period of the study. Individual body weights were measured at the initiation of the test (Day 0), at day 5 and at termination of the test on Day 8. The total estimated food consumption during the five-day exposure period was determined for treatment and control groups.

RESULTS

No mortality occurred in the control or in the treatment group.

Validity criteria according to the current OECD guideline 205 (1984):
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%).
- The lower test item concentration (5000 ppm) resulted in no mortality and no observable toxic effects.

The following validity criterion was not met:

- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (no analytical results were reported).

Some data are missing in order to be able to conclude on the achievement of the validity criteria and there were deviations to the guideline recommendation, so the study was proposed to be supportive only.

CONCLUSIONS

Assessment and conclusion by applicant:
The dietary LC_{50} and NOEC for Japanese quails exposed to glyphosate technical were determined to be > 5000 ppm (nominal), which is calculated to be equivalent to LD_{50}>1341.6 mg a.s./kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:
The study has not been evaluated in detail by the RMS. However, based the limitations pointed out by the GRG, especially those related to the validity criteria of OECD TG 205, the study should not be used further for the risk assessment.

Data point	CA 8.1.1.2/04
Report author	[redacted]
Report year	1973
Report title	Eight-day dietary LC_{50} – Mallard ducks technical CP67573
Report No.	241-107
Document No.	-
Guidelines followed in study	Not mentioned
Deviations from current test guideline identified by the applicant:	The acclimation period duration is not reported (min of 7 days is required)

See RMS analysis in RMS comment box	Mortality during 72h before exposure should be assessed in order to validate the batch of organisms (not reported) The spacing factor between concentrations should not exceed 2 (2.15 was used) Bodyweights not determined for Day 5, only day 0 and day 8 reported.
Previous evaluation	Yes, evaluated and accepted in Monograph 2001
GLP/Officially recognised testing facilities	No, not reported in the report
Acceptability/Reliability	Not acceptable, did not fulfil validity criteria. See below.

MATERIAL AND METHODS

Test material:	Technical CP67573
Lot/Batch #:	LH 14, 583A
Purity:	Assumed to be 100% (98.5%)
Vehicle and/or positive control:	Corn oil included in the basal diet Positive control: Dieldrin
Test organism:	
Species:	Mallard duck (*Anas platyrhynchos*)

STUDY DESIGN AND METHODS

Treatments:	The test consisted of a geometric series of five test concentrations for the test and the toxic reference items and a control group. Fifty mallard duck birds were randomly assigned to the control group and ten birds were randomly assigned to each of the treated groups. The birds were housed in brooding pens containing ten birds each. The nominal concentrations of Technical CP67573, administered by food mixing, were 215, 464, 1000, 2150 and 4640 ppm a.s. Diets were presented to the birds at initiation of the test.
Observations:	Test birds were observed daily. A record was maintained of all signs of toxicity, abnormal behaviours and mortality. Individual body weights were measured at the initiation of the test (Day 0) and at termination of the test on Day 8. The total estimated food consumption during the five-day exposure period was determined for each treatment group and the control group.

RESULTS

No mortality occurred in the control or treatment groups.

<u>Validity criteria according to the current OECD guideline 205 (1984):</u>
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%)
- The lower test item concentration resulted in no mortality and no observable toxic effects.

The following validity criterion was not met:

- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (no analytical results were reported).

Some data are missing in order to be able to conclude on the achievement of the validity criteria and there were minor deviations to the guideline recommendation, so the study is considered as supportive only.

CONCLUSIONS

Assessment and conclusion by applicant:

The dietary LC_{50} and NOEC for mallard ducks exposed to Technical CP67573 were determined to be > 4640 ppm (nominal), which is calculated to be equivalent to LD_{50} > 1103.9 mg a.s./kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:

The study has not been evaluated in detail by the RMS. However, based the limitations pointed out by the GRG, especially those related to the validity criteria of OECD TG 205, the study should not be used further for the risk assessment.

Data point	CA 8.1.1.2/05
Report author	[redacted]
Report year	1997
Report title	Glyphosate acid: Dietary LC_{50} to the mallard duck
Report No.	ZCA 23
Document No.	-
Guidelines followed in study	EPA FIFRA 71-2 (1982) and OECD 205 (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	None compared with OECD 205 (1984) according to the applicant.
Previous evaluation	Submitted but not used in RAR 2015
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Acceptable

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6%
Vehicle and/or positive control:	Not required Positive control: none
Test organism:	

Species:	Mallard ducks (*Anas platyrhynchos*)
Age:	Ten days old at test start
Number of animals:	10 birds per pen; 2 pens in control group; 5 pens in treatment
Weight:	120 - 131 g (at test initation)
Source:	[redacted]
Food:	Standard HRC chick diet *ad libitum* during acclimation and during the test.
Acclimation period:	3 days
Environmental conditions:	
Temperature:	26 - 29 °C
Relative humidity:	72%
Photoperiod:	10 hours light / 14 hours darkness

STUDY DESIGN AND METHODS

Treatments:	The dietary toxicity test was performed by feeding a series of 5 nominal dietary test doses encompassing; 325, 650, 1300, 2600 and 5200 mg test item/kg diet, prepared by mixing the test item with untreated diet. In addition, two control groups were fed with untreated diet only. One pen containing 10 test birds was used per dose group. For the control, two pens containing 10 birds each were used. The birds were exposed for 5 days to the treated diet.
Observations:	Birds were observed daily throughout the study and at defined intervals during the treatment and post treatment periods. Mortality, bird health and clinical signs including appearance of excreta were recorded at each observation. Body weights by group were measured three days prior to exposure, on day 0 (immediately prior to introduction of test diet), day 5 and day 8. Mean group food consumption was recorded at time intervals, from day -3 to -1 and 5 to 8 and daily from days 0 to 4. At test termination, macroscopic post mortem examination was carried out on ten birds of the highest surviving dose group and ten from the control. Tissue examination included gastrointestinal tract, liver, kidney, heart, spleen, muscle and subcutaneous fat. The birds were observed for any gross pathological changes.
Analytical measurements:	Yes, by means of HPLC. The analytical method was not evaluated in detail by the RMS, but is regarded as 'fit for purposes' since this is a vertebrate study.
Statistical analysis:	Not reported. No mortality occurred.

RESULTS

Clinical signs and mortalities: All birds remained in good health throughout the study and there were not mortalities in either the control or treatment groups.

Body weight: Body weight changes were variable in all groups and there was no evidence of any treatment-related effect.

Feed consumption: Food consumption was similar in all groups and there was no evidence of any treatment-related effects.

Macroscopic post mortem examination: No abnormalities were detected in any birds during post mortem examination at termination of the study.

Table B.9.1.1.2-3: Summary of effects of glyphosate acid on mortality of mallard duck (*Anas platyrhynchos*) following dietary oral exposure

Treatment (ppm)	8-day cumulative mortality [No. dead/No. exposed]	Mean body weight day 0 [g]	Mean body weight day 5 [g]	Mean food consumption over 5 days [g/bird/day]
Control (0)	0/10	131	259	65
Control (0)	0/10	126	252	61
Glyphosate (325)	0/10	126	257	63
Glyphosate (650)	0/10	124	256	60
Glyphosate (1300)	0/10	120	239	58
Glyphosate (2600)	0/10	125	247	61
Glyphosate (5200)	0/10	126	256	63

There were no mortality and sign of clinical toxicity observed in any bird. In addition, body weight and food consumption remained unaffected by treatment with glyphosate acid. At necropsy, no abnormalities were observed.

Validity criteria according to the current OECD guideline 205 (1984):
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%)
- The lower test item concentration resulted in no mortality and no observable toxic effects.
- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (actual was between 100.5 and 106.0 %).

CONCLUSIONS

Assessment and conclusion by applicant:
The dietary LC_{50} and NOEC for mallard duck exposed to glyphosate acid were determined to be > 5200 ppm (nominal), which is calculated to be equivalent to LD_{50} > 1715.2 mg a.e./kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:
The study was not evaluated in detail by the RMS, however, although some minor deviations were noted it seems to comply with the relevant validity criteria. There is no indication that dietary exposure to glyphosate is more severe than from the acute oral exposure. The study is considered as acceptable. The results are used to confirm the low toxicity concluded from the available acute oral data.

Data point CA 8.1.1.2/06

Report author	[redacted]
Report year	1991
Report title	AMPA: A Dietary LC_{50} Study with the Northern Bobwhite.
Report No.	139-275
Document No.	-
Guidelines followed in study	FIFRA 71-2 and ASTM Standard E857-81
Deviations from current test guideline identified by the applicant:	Mortality during 72h before exposure should be assessed in order to validate the batch of organisms (not reported but birds appeared to be in good health at initiation of the test).
See RMS analysis in RMS comment box	Relative humidity lower than 50-75 recommended (actual 41 ± 1°%).
Previous evaluation	Yes, evaluated and accepted in Monograph 2001
GLP/Officially recognised testing facilities	Yes, conducted under GLP officially recognised testing facilities
Acceptability/Reliability	Not acceptable, validity criteria not met. See below.

MATERIAL AND METHODS

Test material:	AMPA
Lot/Batch #:	PIT-9008-2407T
Purity:	97% (87.8% analysed after the test completion)
Vehicle and/or positive control:	Corn oil included in the basal diet Positive control: none
Test organism:	
Species:	Northern bobwhite, Bobwhite quail (*Colinus virginianus*)

STUDY DESIGN AND METHODS

Treatments:

The test consisted of a geometric series of five test concentrations and a control group. Thirty northern bobwhite chicks were randomly assigned to the control group and ten northern bobwhite chicks were randomly assigned to each of the treatment groups.
The nominal concentrations, administered by food mixing, were; 562, 1000, 1780, 3160 and 5620 ppm AMPA. Diets were presented to the birds at initiation of the test, following a five-day acclimation period.

Observations:

During acclimation all birds were observed daily. Test birds were observed twice daily throughout the remainder of the test. A record was maintained of all signs of toxicity and abnormal behaviours.
Individual body weights were measured at the initiation of the test (Day 0), on Day 5 and at termination of the test on Day 8. Average feed consumption values were determined daily during the exposure period (Days 0-5) and daily during the post-exposure observation period (Days 6-8) by pen for each treatment group and the control group.

RESULTS

No mortality was observed in the control or treatment groups, except for a single mortality on day 2 at the 562 ppm test concentration which was not considered treatment related. There were no mortalities in any of the other test concentrations and all other birds were normal in appearance and behaviour throughout the study. When compared to the controls, there was no effect on body weight gain or feed consumption.

Validity criteria according to the current OECD guideline 205 (1984):
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%).
- The lower test item concentration resulted in no mortality and no observable toxic effects.

The following validity criterion was not met:

- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (analytical results were not reported).

Some data are missing in order to be able to conclude on the achievement of the validity criteria and birds' batch acceptability criteria.

CONCLUSIONS

Assessment and conclusion by applicant:
The dietary LC_{50} and NOEC for northern bobwhite exposed to AMPA were determined to be > 5620 ppm (nominal), which is calculated to be equivalent to LD_{50} >2161.5 mg AMPA/kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:
The study has not been evaluated in detail by the RMS. However, based the limitations pointed out by the GRG, especially those related to the validity criteria of OECD TG 205, the study should not be used further for the risk assessment.

Data point	CA 8.1.1.2/07
Report author	[redacted]
Report year	1991
Report title	AMPA: A Dietary LC_{50} Study with the Mallard.
Report No.	139-276
Document No.	-
Guidelines followed in study	FIFRA 71-2 and ASTM Standard E857-81
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Mortality during 72h before exposure should be assessed in order to validate the batch of organisms (not reported but birds appeared to be in good health at initiation of the test)
Previous evaluation	Yes, evaluated and accepted in Monograph 2001
GLP/Officially recognised testing facilities	Yes, conducted under GLP officially recognised testing facilities
Acceptability/Reliability	Not acceptable, validity criteria not met. See below.

MATERIAL AND METHODS

Test material:	AMPA
Lot/Batch #:	PIT-9008-2407T
Purity:	97% (87.8% analysed after the test completion)
Vehicle and/or positive control:	Corn oil included in the basal diet Positive control: none
Test organism:	
Species:	Mallard duck (*Anas platyrhynchos*)

STUDY DESIGN AND METHODS

Treatments:	The test consisted of a geometric series of five test concentrations and a control group. Thirty northern bobwhite chicks were randomly assigned to the control group and ten northern bobwhite chicks were randomly assigned to each of the treatment groups. The nominal concentrations, administered by food mixing, were 0, 562, 1000, 1780, 3160 and 5620 ppm AMPA. Diets were presented to the birds at initiation of the test, following a five-day acclimation period.
Observations:	During acclimation all birds were observed daily. Test birds were observed twice daily throughout the remainder of the test. A record was maintained of all signs of toxicity and abnormal behaviours. Individual body weights were measured at the initiation of the test (Day 0), on Day 5 and at termination of the test on Day 8. Average feed consumption values were determined daily during the exposure period (Days 0-5) and daily during the post-exposure observation period (Days 6-8) by pen for each treatment group and the control group.

RESULTS

No mortality was observed in the control or treatment groups. One bird at the 3160 ppm concentration was noted with a leg injury on Day 5. All other birds were normal in appearance and behaviour throughout the study. A reduction in body weight gain and feed consumption was noted at the 3160 ppm concentration from Day 0 to Day 5, it was not considered treatment related as there was no effect at the higher concentration.

<u>Validity criteria according to the current OECD guideline 205 (1984):</u>
The following validity criteria were met:

- The mortality in the control group did not exceed 10% (actual value: 0%)
- The lower test item concentration resulted in no mortality and no observable toxic effects.

The following validity criterion was not met:

- Concentrations of the test substance in the diet should be maintained (at least 80% of nominal) throughout the exposure period (analytical results were not reported).

Some data are missing in order to be able to conclude on the achievement of the validity criteria and birds' batch acceptability criteria.

CONCLUSIONS

Assessment and conclusion by applicant:
The dietary LC_{50} and NOEC for mallard duck exposed to AMPA were determined to be > 5620 ppm (nominal), which is calculated to be equivalent to LD_{50} > 1765.2 mg AMPA/kg bw/day.

This study is considered as supportive and will not be used in the risk assessment.

Assessment and conclusion by RMS:
The study has not been evaluated in detail by the RMS. However, based the limitations pointed out by the GRG, especially those related to the validity criteria of OECD TG 205, the study should not be used further for the risk assessment.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Data point	CA 8.1.1.3/001
Report author	[redacted]
Report year	1999
Report title	Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*)
Report No.	123-186
Document No.	-
Guidelines followed in study	FIFRA Guideline 71-4; OECD Guideline 206
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	No deviations compared to OECD Guideline 206.
Previous evaluation	Yes, but not considered acceptable in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	P24 (information from applicant, not stated in the study report)
Purity:	95.6%
Vehicle and/or positive control:	None Positive control: none
Test organism:	

Species:	Bobwhite quail (*Colinus virginianus*)
Age:	Young adults, 30 weeks (at test initiation)
Sex:	Males and females
Weight:	196 to 250 g (at test initiation)
Source:	[redacted]
Food:	Game bird ration, *ad libitum*
Acclimation period:	0 weeks
Environmental conditions:	
Temperature:	23.1 ± 1.8°C (adults); 27.3± 1.2°C (hatchling) 38°C (brooding compartment)
Humidity:	66 ± 12% (adults); 40 ± 17% (hatchling)
Photoperiod:	17 hours light / 7 hours dark, (approx. 265 lux)

STUDY DESIGN AND METHODS

Replicates:

Sixteen replicates (1 male and 1 female per pen) were used for each treatment group and control.

Loading:

Approx. 0.138 m^2 (27 x 51 cm) for 2 birds (1 males and 1 female per pen), 72 x 90 cm for hatchlings from each brooding pen.

Treatments:

Nominal dietary doses were 500, 1000 and 2250 mg /kg feed. The birds were exposed to the treated diets for approximately 20 weeks.

Eggs were collected daily and stored at 13.6 ± 0.6°C and 82 ± 8% relative humidity. All eggs laid within a week were considered as one lot and incubated in a Petersime Incubator. On day 21 of incubation, eggs were placed in a Petersime Hatcher and allowed to hatch.

The hatchlings were maintained on untreated diet until 14 days of age.

Observations:

Adult birds were observed daily for signs of toxicity and abnormal behaviour throughout the study.

Adult body weight was measured at study initiation and termination, in addition to on weeks 2, 4, 6, and 8. For each pen, food consumption was measured weekly throughout the study except for the last interval, where food consumption was measured over a 6 day period.

At the end of each week, all collected eggs were counted and a single egg was randomly selected for eggshell thickness measurements. The remaining eggs were candled to detect egg shell cracks or abnormal eggs before incubation. During the incubation period, eggs were candled again on day 11 or 12 to evaluate embryo viability and on day 21 to determine embryo survival.

During the study, total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survivorship of 14 day old hatchlings were determined.

Analytical measurements: Homogeneity of the test substance in treated diets was evaluated by collecting 6 samples of each treatment group on day 0 of week 1. During weeks 2, 3, 4, 8, 12, 16 and 20 of the test, a single sample was collected from the control diet and an additional duplicate sample was collected from treatment group diet, to measure and/ or verify test concentrations. The analytical method was assessed by the RMS as Valid and 'fit for purpose'.

Statistical analysis: An analysis of variance (ANOVA) was used to determine significant differences among the groups followed by Dunnett's multiple comparison procedure as the post-hoc test.

RESULTS

Analysis of samples resulted in measured concentrations of 100%, 99% and 96% of the nominal test doses of 500, 1000 and 2250 mg glyphosate acid/kg feed, respectively.

No treatment-related mortality of parental birds exposed to glyphosate acid was observed. No overt symptoms of toxicity or treatment related effects upon body weight or feed consumption were observed at any dietary dose tested. In addition, no treatment-related effects of reproductive parameters were observed at any dose tested.

Table B.9.1.1.3-1: Effects of glyphosate acid on reproductive performance of bobwhite quail over 8 weeks, ie. results before exceedance of the validity criterium for adult mortality in the control

Glyphosate acid [mg a.s./kg feed]	Control	500	1000	2250
Replicates (start/8 w exposure)	16	16	16	16
Reproductive performance				
Number of eggs laid per female [mean]	37	36	33	37
Eggs laid/maximum laid [%]	64	63	57	64
Eggs cracked/egg laid [%]	3	2	8	2
Viable embryos/egg set [%]	87	92	95	94
Live 3-week embryos/viable embryos [%]	100	99	99	99
Hatchlings/live 3-week embryos [%]	96	96	96	97
14-day-old survivors/hatchlings [%]	94	97	95	96
Hatchlings/egg set [%]	83	87	90	90
14-day-old survivors/egg set [%]	77	85	86	86
Hatchlings/maximum set [%]	48	51	45	53
14-day-old survivors/ maximum set [%]	44	50	43	51

Table B.9.1.1.3-2: Results over 10 weeks. Note that by the end of the study, adult mortality in the control exceeded the criteria for validity according to OECD 206

Glyphosate acid [mg a.s./kg feed]	Control	500	1000	2250
Replicates (end of exposure period at 10 weeks)	10	14	14	14
Reproductive performance				
Number of eggs laid per female [mean]	47.7	42.2	39.0	44.0
Eggs laid/maximum laid [%]	70	62	57	65
Eggs cracked/egg laid [%]	5	4	11	5

Viable embryos/egg set [%]	81	91	94	92
Live 3-week embryos/viable embryos [%]	99	98	98	98
Hatchlings/live 3-week embryos [%]	95	95	95	97
14-day-old survivors/hatchlings [%]	93	96	95	96
Hatchlings/egg set [%]	75	85	88	88
14-day-old survivors/egg set [%]	70	82	83	85
Hatchlings/maximum set [%]	50	51	44	54
14-day-old survivors/ maximum set [%]	46	49	43	52
Eggshell thickness				
Mean shell thickness [mm]	0.220	0.228	0.222	0.216
Body weight of hatchling				
Mean body weight [g]	6 ±0	6 ±0	7 ±1	6 ±1
Body weight of 14-day old survivors				
Mean body weight [g]	26 ±3	28 ±2	28 ±2	27 ±2

Table B.9.1.1.3-3: Effects of glyphosate acid on adult bodyweight and feed consumption of adult bobwhite quail

Glyphosate acid [mg a.e./kg feed]		**Control**	**500**	**1000**	**2250**
Average body weight [g]					
Test initiation	M	215	223	216	214
	F	219	219	216	218
After 18 weeks	M	n.r.	n.r.	n.r.	n r.
	F	n.r.	n.r.	n.r.	n r.
Test termination	M	219	229	219	215
	F	250	248	238	239
Body weight change (test start - test end)	M	4	6	3	2
	F	31	29	21	23
Average feed consumption [g/bird/day]					
Week 1	M + F	12	12	12	12
Week 5	M + F	12	12	12	13
Week 10	M + F	19	18	19	20
Week 15	M + F	26	26	26	28
Week 18	M + F	26	26	27	27
Week 20	M + F	25	26	25	26

M = male, F = female

The mortality of the control exceed 10% at the end of the test (actual value: 6 of the 32 birds were found dead). This did not seem to affect the average number of 14-day-old survivors per hen in the control, which was greater than 12. Also, the average egg shell thickness for the control group was greater than 0.19 and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

CONCLUSIONS

Assessment and conclusion by applicant:

The NOEL for bobwhite quail exposed to glyphosate acid in a reproduction study was determined to be 2250 mg glyphosate acid/kg feed (based on nominal doses).

The NOEL for bobwhite quail exposed to glyphosate acid in a reproduction study was determined to be 2250 mg/kg feed (201 mg kg bw/d) and can be used in risk assessment.

Although the control mortality exceeded 10% at the end of the test, the study is still considered valid. A letter (CA 8.1.1.3/002) from [redacted] where this study was conducted, provides additional justification regarding the observed mortalities. It is indicated that a 'hysteria attack' occurred and the birds obtained serious injuries due to this and were not treatment related. The control performance from this study were compared with historical control data (from 21 studies) from the laboratory which shows that there was no significant difference.

The cage size used in this bobwhite study has been previously criticised and is also addressed in this letter from the laboratory. The cage size is acceptable based on the fact that the reproductive performance of the studies are good and that both control and treated birds are housed in the same way without high mortality levels and therefore this is not a potential contributing factor for the control mortality observed in this study.

Assessment and conclusion by RMS:

The former RMS for the previous evaluation of glyphosate identified several shortcomings of this study (see RAR 2015):

Age of the test organisms: According to OECD 206 the age of the test organisms should range between 20-24 weeks. Adult bobwhite quail birds in the present study were 30 weeks.

Loading: The test guideline recommends a minimum floor area of pen per pair of 0.25 m^2, whereas in the present study the loading was 2 birds on 0.138 m^2, probably leading to unfavourable conditions to test animals.

Validity criteria: The mortality in the controls should not exceed 10 % at the end of the test. At start of week 19, 12 mortalities occurred, all of which were hens. Of the 12 mortalities, six occurred in the control group and two occurred in each of the three treatment groups, probably due to limited space leading to stress. It is noted that a mass cannibalistic event (hysteria) is less likely to do as much damage when birds are housed farther apart, as weaker birds are more able to avoid aggressive birds.

Hence, the former RMS considered that the study is not acceptable or valid. It is also noted that no acclimation period was used (at least 2 weeks according to OECD TG 206).

The applicant has provided further justification to support the validity of the results (see [redacted] 2013, summarized below). Given that an egg collection period shorter than the recommended 10 weeks (OECD 206) is normally accepted for this type of study, it seems reasonable to rely on the results from before the observed deaths (8 weeks) in the control in order to meet the validity criteria. Therefore, these values were included in the result table above.

Endocrine disruption: In the gross pathological examination at test termination, increased regressing and/or regressed ovaries were observed at 1000 and 2500 ppm (3 out of 14 birds at 1000 ppm, 1 out of 14 birds at 2500 ppm). In male animals, at 1000 ppm elevated numbers of small testes were

observed (2 compared to 1 in the control). This effect was not observed in the highest does of 2500 ppm treatment. The findings were considered by the authors to be incidental to treatment.

Data point	CA 8.1.1.3/002
Report author	[redacted]
Report year	2013
Report title	Letter concerning the study; Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*). Study report 123-186
Report No.	letter regarding 123-186
Document No.	-
Guidelines followed in study	-
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	-
Previous evaluation	-
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability	Yes

The applicant has included a summary of a letter provided by study director at the performing laboratory concerning the study; Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (Colinus virginianus). Study report 123-186.

Although the control mortality exceeded 10% at the end of the test, it was proposed that the study should still be considered as valid. It is indicated that a 'hysteria attack' occurred and the birds obtained serious injuries due to this and were not treatment related. The control performance from this study were compared with historical control data (from 21 studies) from the laboratory which shows that there was no significant difference.

The cage size used in this bobwhite study has been previously criticised and is also addressed in this letter from the laboratory. It was proposed that the cage size is acceptable based on the fact that the reproductive performance of the studies is good and that both control and treated birds are housed in the same way without high mortality levels and therefore this is not a potential contributing factor for the control mortality observed in this study.

CONCLUSIONS

Assessment and conclusion by RMS:

Agree that the adult mortality in the control does not necessarily invalidate the study in this case, since the mortalities occurred late and there are still results from 8 weeks of egg production before the mortality events. Concerning loading of birds, from our experience this is generally not regarded as a crucial factor for acceptability of a study, especially in case there is no obvious impact on reproductive parameters compared to control data from other studies. Therefore, although the pen size is smaller than the recommended by OECD 206, and age of the birds were outside of the recommended range, our overall conclusion is that the study by [redacted] 1999 can be accepted.

The evaluation against historical control data was not presented in detail, and therefore it is not possible to confirm whether the conditions for these data are comparable to the current study.

It is noted that the NOEL (201 mg a.s./kg bw per day) from the study by [redacted] 1999 is not critical for the risk assessment.

Data point	CA 8.1.1.3/003
Report author	[redacted]
Report year	1978
Report title	One-Generation Reproduction Study – Bobwhite Quail; Glyphosate Technical
Report No.	139-141
Document No.	-
Guidelines followed in study	Non-stated
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	No major deviations from OECD guideline 206. Parental mortality data was not reported.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No
Acceptability/Reliability	Not acceptable, did not fulfil validity criteria. See RMS comments below.

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	XHI 162
Purity:	83% (measured)
Vehicle and/or positive control:	Corn oil Positive control: none
Test organism:	
Species:	Bobwhite quail (*Colinus virginianus*)
Age:	5 months old (young adults)
Sex:	Males and females
Weight:	Not stated
Source:	In-house production flock
Food:	Game bird breeder ration, *ad libitum*
Acclimation period:	Not stated (in-house)
Environmental conditions:	
Temperature:	21.1 – 26.7°C (research facility) 15.6°C (eggs storage), 37.4 - 37.6°C (eggs incubation)
Humidity:	55% (eggs storage)
Photoperiod:	9 hours light / 15 hours dark (first 6 weeks) 17 hours light / 7 hours dark (following 16 weeks)

STUDY DESIGN AND METHODS

Replicates: Twelve replicates (with 1 male and 2 females per pen per replicate) were exposed per treatment group and control.

Loading: 1 males and 2 females per pen (pen size not reported)

Treatments: Nominal dietary doses were 50, 200 and 1000 mg glyphosate acid/kg diet. The diet was prepared by incorporating appropriate concentrations of the test item and corn oil into the aliquots of basal diet.

The birds were exposed for nine weeks to the treated diet prior to egg deposition and for additional eight weeks during egg collection. Eggs were collected daily, stored at 15.6°C and 55% relative humidity and were cleaned weekly. The eggs were then incubated at 37.5 ± 0.06°C.

On day 19 of incubation, the eggs were placed in a Humidaire hatcher and allowed to hatch. All hatchlings were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age.

Observations: Body weights were stated to be recorded at study initiation, 5 weeks after study initiation prior to onset of egg deposition and at termination of the study. Food consumption was recorded every second week throughout the study.

All eggs were candled on day 0 of incubation for eggshell cracks, on day 14 to measure embryo viability, and on day 19 to measure embryo survival. Weekly throughout the egg deposition period, one egg of each pen in each group was randomly selected for egg weight and eggshell thickness measurement.

During the study total egg production, number of eggs cracked, egg set, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 days-old hatchlings, 14 day survival, egg weight and eggshell thickness were determined.

Analytical measurements: Not reported.

Statistical analysis: To evaluate differences between reproductive parameters, Student's t-test was used.

RESULTS

Adult mortality, body weight, food consumption, and possible sublethal effects other than reproduction were not presented in the study report.

Table B.9.1.1.3-4: Effects of glyphosate on reproductive parameters of bobwhite quail

Glyphosate acid [mg a.e./kg diet]	Control	50	200	1000

Replicates	12	12	12	12
Reproductive success				
Total number of eggs laid per group in 8 weeks	764	673	673	781
Number of eggs laid per hen in 8 weeks (mean)**	31.9	28.0	28.0	32.5
Number of eggs cracked [%]	9.7	7.6	9.2	6.3
Viable embryos of egg set	91.3	80.7	91.7	87.0
Live 3-week embryos of viable embryos [%]	97.3	97.2	97.5	96.5
Hatchlings of live 3-week embryos [%]	81.5	70.3	73.4	74.4
14-day-old survivors of normal hatchlings [%]	95.5	93.1	95.7	93.5
14- day-old survivors per hen [a]	18.7	12.3	14.8	16.7
Egg weight				
Mean egg weight [g]	10.3	9.9	10.2	9.4 *
Eggshell thickness				
Mean eggshell thickness [mm]	0.214	0.204	0.211	0.224
Body weight of representative hatchling				
Mean body weight [g]	6.8	6.9	6.9	6.7
Body weight of representative 14-day old survivors				
Mean body weight [g]	22.0	22.2	22.6	22.0

[a] based on 24 hens
* Statistically significant compared to control (Student's t-test)
**added by the applicant assuming no mortality, not from the original study

A statistically significant reduction in egg weight occurred at the highest test dose of 1000 mg glyphosate acid/kg di*et. al*though there was a small reduction in egg weight at 1000 mg/kg feed there was not a significant impact on the biologically relevant endpoints that included initial hatchling body weight, 14 day hatchling body weight, egg shell thickness and hatchling survival.

According to the applicant, all current validity criteria were fulfilled, as the mortality of the control was claimed not to exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was ≥ 14. Also, the average egg shell thickness for the control group was ≥ 0.34 mm and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

CONCLUSIONS

Assessment and conclusion by applicant:
Based on the overall results of this study, the NOEL for bobwhite quail exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg glyphosate acid/kg diet.

This study is considered valid and the NOEL for bobwhite quail exposed to glyphosate acid in a one-generation reproduction study was determined to be 1000 mg/kg diet (96.3 mg/kg bw/d) and can be used in risk assessment.

Assessment and conclusion by RMS:
According to the methods description, adult body weight and food consumption were recorded within the study. However, the results from these measurements are not included in the test report. It is possible that some pages are missing in the presentation of results. Due to the lack of these data, it is unclear how the endpoint based on daily dose was calculated.

Adult mortality was not recorded. All results are reported per replicate with two hens and one drake, therefore there is no data on mortality at individual level. Given the number of eggs laid per replicates in the control, it seems likely that at least 3 replicates (with much lower egg numbers) may have lost one of their two egg producers. Hence, it is not possible to confirm whether the validity criterium of

<10% control mortality is met. Further, the calculated mean values per hen presented in the study summary above seemingly presumes that no mortality occurred in any replicate, although this was not clear from the study report.

Age of birds was within acceptable range from OECD 206.

Exposure duration in this study (9 + 8 weeks) was not consistent with OECD 206, where 20 weeks (egg collection during 10 weeks) is recommended. An egg collection period of 8 week is however normally accepted.

Cage size and floor area per bird were not reported. Therefore, it is unclear whether the loading recommendations of OECD 206 were met.

No analytical measurements were made to verify the nominal exposure levels. Lack of measured test concentrations must be regarded as a drawback since it is not known whether the actual treatment levels were within 80-120% as recommended by OECD 206.

Agree that the observed statistically significant effect on egg weight at the highest test concentration should be regarded as not biologically significant, since there was no corresponding reduction in growth and development of the hatchlings.

Overall, given that the validity of the study could not be confirmed (adult mortality not reported), that the conversion of the endpoint to daily dose was unclear (no data recorded on adult body weight and food consumption), and the lack of verification of the treatment levels, this study is not considered as valid.

It is noted that the previously agreed NOEL from this study was the most conservative endpoint for birds. However, since the study is no longer consider valid, the reproductive endpoint is based on the endpoint derived from [redacted] 1999.

Data point	CA 8.1.1.3/004
Report author	[redacted]
Report year	1999
Report title	Glyphosate Acid: A Reproduction Study with the Mallard (*Anas platyrhynchos*)
Report No.	123-187
Document No.	-
Guidelines followed in study	FIFRA Guideline 71-4 OECD Guideline 206
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	No deviations from OECD Guideline 206
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate acid
Lot/Batch #:	P24
Purity:	95.6%
Vehicle and/or positive control:	Vehicle: None Positive control: none
Test organism:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	21 weeks (at test initiation)
Sex:	Males and females
Weight:	868 to 1259 g (at test initiation)
Source:	[redacted]
Food:	Game bird ration, *ad libitum*
Acclimation period:	6 weeks
Environmental conditions:	
Temperature:	22.4 ± 0.9°C (adults); 29 °C (hatchling); 38°C (brooding compartment)
Humidity:	69 ± 13% (adults); 61 ± 15% (hatchling)
Photoperiod:	7-8 hours light per day for the first 10 weeks 17 hours light / 7 hours dark during egg laying, (approx. 292 Lux)

STUDY DESIGN AND METHODS

Replicates:

Sixteen replicates (1 male and 1 female per pen, 16 pen per treatment group) were used for each treatment group and control.

Loading:

Approx. 0.675 m^2 for 2 birds (1 males and 1 female per pen)

Treatments:

Nominal dietary doses were 500, 1000, and 2250 mg a.e./kg feed.
The birds were exposed to the treated diets for approximately 21 weeks, and were evaluated for treatment-related effects on bird health and reproduction.

Eggs were collected daily, washed and stored in a cold room at 13.6 ± 0.6°C and 82 ± 8% relative humidity.

All eggs laid within a week were considered as one lot and were incubated in a Petersime incubator. On day 24 of incubation, eggs were placed in a Petersime hatcher and were allowed to hatch.

The hatchlings were maintained on untreated diet until 14 days of age.

Observations:

Parental birds were observed daily throughout the study for signs of toxicity and abnormal behaviour.

Adult body weights were measured at study initiation and termination in addition to on weeks 2, 4, 6, and 8 of the adult

in-life period. For each pen, feed consumption was measured weekly.

At the end of each week, all eggs collected were counted and selected by indiscriminate draw for eggshell thickness measurement. The remaining eggs were candled to detect egg shell cracks or abnormal eggs before incubation. During the incubation period, eggs were candled again on day 14 to investigate embryo viability and on day 21 to determine embryo survival.

During the study, total egg production, number of eggs cracked, eggshell thickness, embryo viability, embryo survival, number of hatchlings, body weight of new hatchlings, body weight of 14 day old hatchlings and survival of hatchlings after 14 days were determined.

Analytical measurements: Homogeneity of the test substance in treated diet was evaluated by collecting 6 samples from each treatment group on day 0 of week 1. During weeks 2, 3, 4, 8, 12, 16 and 20 of the test, a single sample was collected from the control diet and an additional duplicate sample was collected from treatment group diet, to measure and/ or verify test concentrations. The analytical method was assessed by the RMS as Valid and 'fit for purpose'.

Statistical analysis: An analysis of variance (ANOVA) was used to determine significant differences among the groups and Dunnett's multiple comparison procedure was used as post-hoc test.

RESULTS

Analytical recovery of the test item ranged from 85 to 119% throughout the study. Therefore, calculated endpoints will be based on nominal concentrations.

Table B.9.1.1.3-5: Concentrations of glyphosate acid in treated diets

Nominal concentration of glyphosate acid [mg a.s./kg feed]	**Control**	**500**		**1000**		**2250**	
Day 0 (% of nominal)		520 (104)		1010 (101)		2250 (100)	
Day 2 (% of nominal)	< 20	481 (96)	476 (95)	927 (93)	945 (95)	1990 (88)	2210 (98)
Day 3 (% of nominal)	< 20	465 (93)	455 (91)	947 (95)	973 (97)	2040 (91)	2130 (95)
Day 4 (% of nominal)	< 20	465 (93)	473 (95)	935 (94)	957 (96)	1990 (88)	2220 (99)
Day 8 (% of nominal)	< 20	478 (96)	469 (94)	938 (94)	848 (85)	2010 (89)	2040 (91)
Day 12 (% of nominal)	< 20	523 (105)	568 (114)	1030 (103)	1040 (104)	2220 (98)	2230 (99)
Day 16 (% of nominal)	< 20	586 (117)	544 (109)	1090 (109)	1190 (119)	2510 (112)	2220 (99)

Day 20 (% of nominal)	< 20	523 (105)	512 (102)	1000 (100)	999 (100)	2190 (97)	2200 (98)

There were no treatment related mortalities at any of the concentrations. However, three incidental adult mortalities occurred during the course of the study. One incidental mortality occurred in the control group and in both the 500 and 1000 mg a.s./kg feed treatment groups. Except for incidental clinical findings, all birds appeared normal throughout the study. Clinical signs as lameness and wing droop were observed and frequently were associated with the incidental injuries.

Table B.9.1.1.3-6: Effects of glyphosate acid on reproductive performance of mallard duck[1]

Glyphosate acid [mg a.e./kg feed]	**Control**	**500**	**1000**	**2250**
Replicates	15	15	15	16
Reproductive performance				
Number of eggs laid per female [mean]	43.6	40.1	40.2	44.3
Eggs laid/maximum laid [%]	61	56	56	62
Eggs cracked/eggs laid [%]	2	1	1	2
Viable embryo/egg set [%]	73	68	93	81
Live 3-week embryos/viable embryos [%]	98	99	99	99
Hatchlings/live 3-week embryos [%]	91	89	84	88
14-day-old survivors/hatchlings [%]	100	91	98	99
Hatchlings/egg set [%]	66	60	78	72
14-day-old survivors/egg set [%]	65	58	76	71
Hatchlings/maximum set [%]	34	31	43	42
14-day-old survivors/ maximum set [%]	34	30	42	42
Eggshell thickness				
Number of eggs measured	58	59	61	65
Mean shell thickness [mm]	0.388	0.374	0.373	0.376
Body weight of hatchling				
Number of juvenile ducks weighted	329	302	414	440
Mean body weight [g]	36	34	35	34
Number of 14 day old survivors	327	291	409	436
Mean body weight [g]	262	236	260	235*

[1] values represent pen means for experimental groups
*significantly different from the control at $p<0.05$

Table B.9.1.1.3-7: Effects of glyphosate acid on adult bodyweight and feed consumption of adult mallard duck

Glyphosate acid [mg a.e./kg feed]		**Control**	**500**	**1000**	**2250**
Average body weight [g]					
Test initiation	male	1091	1103	1106	1107
	female	1024	1021	1019	999
14-day	male	1075	1079	1097	1078
	female	1005	1011	998	983
Test termination	male	1161	1105	1134	1088
	female	1114	1104	1112	1080
Body weight change (test start – test end)	male	68 (+6%)	0 (+0%)	27 (2%)	-19 (-2%)
	female	99 (+10%)	76 (+7.4%	90 (+8.8%)	81 (+8.1%)
Average feed consumption [g/bird/day]					
Week 1		89	102	86	93
Week 5		95	93	92	101
Week 10		137	125	117	127
Week 15		193	193	168	198
Week 21		169	167	170	173

There were no treatment related effects upon reproductive performance at any of the concentrations tested. However, offspring in the 2250 ppm treatment group did show a slight, but statistically significant

($p<0.05$) reduction in the mean body weight of 14-day old survivors when compared to the control. The mean body weight value for 14 day old survivors in the control group was 262 ± 32 g while mean values for the 500, 1000 and 2250 mg a.s./kg feed treatment groups were 236 ± 35 g, 260 g ± 16 g, 235 g ± 23 g, respectively. As especially the parameters concerning hatchling weight were affected at 2250 mg a.s./kg feed, it cannot be excluded that the observed changes in hatchling weight do not represent a population relevant adverse effect. Therefore, this endpoint will be considered as a NOAEL of 1000 mg a.s./kg feed, corresponding to 116 mg a.e./kg/bw/d.

All validity criteria according to OECD 206 were fulfilled, as the mortality of the control group did not exceed 10 % at the end of the test and the average number of 14-day-old survivors per hen in the control was greater than 14. Also, the average egg shell thickness for the control group was greater than 0.34 and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

CONCLUSIONS

Assessment and conclusion by applicant:
The NOEL for mallard duck exposed to glyphosate acid in a reproduction study was determined to be 2250 mg a.e./kg feed (based on nominal doses).

This study is considered valid and the NOEL for mallard duck exposed to glyphosate acid in a reproduction study was determined to be 2250 mg a.s./kg feed (300 mg a.e./kg bw/day), and can be used in risk assessment.

Assessment and conclusion by RMS:
The RMS agree that the study fulfil the validity criteria of OECD 206.

Due to the slight but statistically significant ($p<0.05$) reduction in the mean body weight of 14-day old survivors when compared to the control a NOAEC of 1000 mg a.e./kg feed, corresponding to 116 mg a.e./kg/bw/d is concluded. This is also consistent with the previous evaluation of the study. The applicant proposed a higher NOEL, assuming that the observed effects on hatchling body weight should not be regarded as population relevant. However, given that the body weight reduction was >10% and statistically significant this was not agreed by the RMS.

Conversion of the dietary endpoint to daily dose was not presented in detail, either in the previous RAR (2015) or in the applicant's summary. According to the RMS' preliminary calculations, the NOAEL corresponds to 116 and 124 mg a.e./kg bw per day for males and females, respectively.

Endocrine disruption: No remarkable effects on ovaries or testes were observed compared to the control in the gross pathological examination at test termination.

Data point	CA 8.1.1.3/005
Report author	[redacted]
Report year	1978
Report title	One-Generation Reproduction Study – Mallard Duck; Glyphosate technical
Report No.	139-143
Document No.	-

Guidelines followed in study	Non-stated
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	No deviations from OECD guideline 206. See also RMS comments below.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	Glyphosate technical
Lot/Batch #:	XHI 162 (Assay of batch as of 6-16-78)
Purity:	83% a.s.
Vehicle and/or positive control:	Corn oil Positive control: none
Test organism:	
Species:	Mallard duck (*Anas platyrhynchos*)
Age:	6 months old (adults, at test initiation)
Sex:	Males and females
Weight:	1047 – 1257 g (at test initiation)
Source:	In-house production flock
Food:	Game bird breeder ration, *ad libitum*
Acclimation period:	Not stated (in-house flock)
Environmental conditions:	
Temperature:	Outdoor temperatures early spring, not controlled. Egg incubation phase; 37.4 – 37.6°C
Humidity:	55% (eggs storage)
Photoperiod:	Outdoor (natural daylight/photoperiod early spring)

STUDY DESIGN AND METHODS

Replicates:	Five replicates (2 males and 5 females per replicate) were used for each treatment group and the control.
Loading:	Approx. 8.2 m^2 for 7 specimens (2 males and 5 females per pen)
Treatments:	Nominal dietary doses of glyphosate technical were 50, 200 and 1000 mg a.s./kg diet. The diet was prepared by incorporating appropriate concentrations of the test item and corn oil into the aliquots of basal diet. The birds were exposed to the treated diet for 9 weeks prior to egg deposition and for additional 8 weeks during egg collection. Eggs were collected daily and stored at 15.6°C and 55% relative humidity and were cleaned weekly. The clean eggs were then incubated at 37.5 ± 0.06°C. On day 22 or 23 of incubation, the eggs were allowed to hatch. The hatchlings

were housed according to the appropriate parental grouping and maintained on control diet until 14 days of age.

Observations:

Body weights were recorded at study initiation, 5 weeks after study initiation, prior to the onset of egg deposition, and at termination of the study. Food consumption was recorded bi-weekly throughout the study.

All eggs were candled on day 0 of incubation for eggshell cracks, on day 14 to measure embryo viability and to remove any E coli-contaminated eggs, and on day 21 to measure embryo survival. Weekly throughout egg deposition period, one egg from each pen in each experimental group and the controls was randomly selected for egg weight and eggshell thickness measurement.

During the study, the total egg production, the number of eggs cracked, embryos viability, embryos survival, number of hatchlings, body weight of representative new hatchling, body weight of representative 14 days-old hatchlings, 14 day-old survivorship, egg weight and the eggshell thickness were determined.

Outdoor temperatures and precipitation were recorded daily.

Analytical measurements:

Not reported.

Statistical analysis:

To evaluate the differences between each of the above-mentioned reproductive parameters, Student's t-test was used.

RESULTS

For the parental birds exposed to glyphosate, no symptoms of toxicity or behavioural abnormalities were recorded at any of the dietary doses tested or the control treatments for the entire test duration. In addition, no mortality was observed in control and treatments groups, except for the highest test dose, at which a single mortality was observed on week 12 after study initiation. This death was however considered incidental, and not compound related.

Table B.9.1.1.3-8: Effects of glyphosate technical on reproductive parameters of Mallard duck

Glyphosate technical [mg a.s./kg diet]	Control	50	200	1000
Replicates (5 females, 2 males each)	5	5	5	5
Reproductive success				
Number of eggs laid per hen in 8 weeks	28	23	28	29
Number of eggs cracked [%]	3	5	5	6
Viable embryos of egg set	90	93	85	86
Live 3-week embryos of viable embryos [%]	96	93	95	95
Hatchlings of live 3-week embryos [%]	74	77	77	81
14-day-old survivors of normal hatchlings [%]	97	99	98	96
14- day-old survivors per hen [a]	16	14	15	16
Egg weight				
Number of eggs analysed	38	38	38	39
Mean egg weight[g]	57.5	58.3	56.3	58.9
Eggshell thickness				

Glyphosate technical [mg a.s./kg diet]	**Control**	**50**	**200**	**1000**
Replicates (5 females, 2 males each)	5	5	5	5
Number of eggs analysed	38	38	38	39
Mean shell thickness [mm]	0.394	0.375	0.372	0.375
Body weight of representative hatchling				
Number of ducklings analysed	72	73	72	73
Mean body weight[g]	33	33	32	34
Body weight of representative 14-day old survivors				
Number of ducklings analysed	72	72	72	73
Mean body weight [g]	217	206	208	205

[a] based on 25 hens

According to the applicant, all validity criteria according to current guidelines were fulfilled, as the mortality of the control did not exceed 10% at the end of the test and the average number of 14-day-old survivors per hen in the control was ≥ 14. Also, the average egg shell thickness for the control group was ≥ 0.34 mm and the lowest treatment level did not result in compound-related mortality or observable toxic effects.

CONCLUSIONS

Assessment and conclusion by applicant:

Based on the results of this study, the NOEL for Mallard duck exposed to glyphosate technical in a one-generation reproduction study was determined to be 1000 mg a.s./kg diet.

This study is considered valid and the NOEL for Mallard duck exposed to glyphosate technical in a one-generation reproduction study was determined to be 1000 mg a.e./kg diet (125.3 mg a.s/kg bw/day) and can be used in risk assessment.

Assessment and conclusion by RMS:

Agree that the validity criteria of OECD 206 are fulfilled, except that no analytical measurements were made to verify the dietary exposure levels of the test substance. Hence, it is not known whether the actual treatment levels were within 80-120% of the nominal as recommended by OECD 206.

Outdoor climate conditions were applied, with daily measurements of temperature and precipitation. Hence, temperature, light and humidity was not controlled during the study. It is not known whether this had an impact, which may add an uncertainty to the results.

Test duration was shorter than recommended by OECD 206, with an egg collection period of 8 weeks, compared to the standard 10 weeks. However, this deviation is normally regarded as acceptable (in line with US EPA; OCSPP 850.2300: Avian reproduction test).

Conversion of the dietary endpoint to daily dose was not presented in detail, either in the previous RAR (2015) or in the applicant's summary. Based on the RMS' preliminary calculation, however, the proposed value (NOEL 125 mg a.e./kg bw per day) seems to be correct.

Endocrine disruption: No gross pathological examination was performed in this study.

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

Please refer to Volume 1, section 2.9.1.4 of this RAR.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

Please refer to Volume 1, section 2.9.1.5 of this RAR.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

Glyphosate acid is stable in water and does not rapidly hydrolyse. Glyphosate has a very low log POW value of <-3.2. Similarly, the main metabolite AMPA is also stable in water and also has a very low log POW value of -2.47. Therefore, as the log POW values for both glyphosate and AMPA are substantially lower than EFSA/2009/1438 trigger value (Log Pow ≥ 3) the potential for bioaccumulation is considered to be low to negligible. The applicant proposed that further consideration of the bioaccumulation potential and food chain behaviour of glyphosate and AMPA is not therefore considered necessary.

This conclusion is supported by the results of a fish bioconcentration study, conducted with bluegill sunfish that achieved a bioconcentration factor (BCF) of 1.1± 0.61, ie. below the Annex VI BCF trigger value of 1000.

Assessment and conclusion by RMS:
The RMS agrees with the conclusion proposed by the applicant. No further data are needed on potential for bioconcentration in prey of birds and mammals.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

See evaluation of open literature data in an Appendix to the RAR for this section.

B.9.1.5. Potential for endocrine disruption

Data point	CA 8.2.3/002
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances
Report No.	707A-103
Document No.	-
Guidelines followed in study	OECD Guideline 231 (2009) OPPTS/OCSPP Guideline 890.1100 (2009)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviations from guideline OECD 231 (2009): Measured test concentrations CV% >20 % due to low recoveries in the low treatment group on Day 14 and in the high treatment group on Day 21.
Previous evaluation	Yes, EFSA ED Conclusion (2017)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability	Yes

MATERIAL AND METHODS

Test material:	MON 77973 (glyphosate acid)
Lot/Batch #:	GLP-1103-21149-T
Purity:	85.14% before drying (95.93% glyphosate acid, dried)
Vehicle and/or positive control:	Dilution water (filtered well water) Positive control: none
Test organism:	
Species:	African clawed frog (*Xenopus laevis*)
Strain:	Not specified
Age at start of dosing:	NF Stage 51, 16 days post-fertilization; all tadpoles were derived from eggs spawned on the same day
Source:	Tadpoles were from eggs collected from adult male frogs and female frogs injected with Hcg induced to spawn in the laboratory; healthy adults obtained from [redacted]
Food:	Sera Micron (Sera North America, PA, USA), 3 times/day
Acclimation period:	Not stated
Aquatic test system:	
Exposure System:	Continuous flow-through diluter system
Flow-through Rate:	69 mL/min
Exposure Vessel:	12 L Glass Aquaria (10 L fill volume)
Source of dilution water:	Filtered fresh well water
Hardness:	142 mg/L as $CaCO_3$ (140 - 144 mg/L as $CaCO_3$)
pH:	8.0 (7.0 – 8.3)
Dissolved Oxygen:	8.2 mg/L (7.6 – 8.7 mg/L)
Iodide:	3 - 6 µg/L
Aeration:	No
Environmental conditions:	
Temperature:	21.9°C (21.4 - 22.3 °C)
Photoperiod:	12 h light/ 12 h dark
Light Intensity at Water's Surface:	911 – 1387 lux

STUDY DESIGN AND METHODS

Replicates:	The number of replicates per treatment was four (4); the number of larvae per replicate per treatment at test initiation was 20 (total: 80 larvae/treatment).
Treatments:	Test concentrations were 0 (dilution water only), 0.16, 0.80, 4, 20, and 100 mg a.s./L. The highest test concentration was selected based on results of a 14-day range-finder and is the guideline-recommended highest test concentration. All test solutions were adjusted for test substance purity.
Observations:	Mortality, Clinical Signs: Survival and clinical signs of toxicity, including any abnormal behavior, were assessed

daily. Dead tadpoles were not replaced in either the control or treatment test chambers.

Developmental Stage: Developmental stage was determined under a dissection microscope based on the developmental stages described by Nieuwkoop and Faber (NF). Developmental stage was determined on Day 7 for five tadpoles randomly selected from each test chamber and on Day 21 for all remaining tadpoles.

Tadpole Growth: Tadpoles were measured for total length to the nearest 1 mm using a metric ruler and were weighed to the nearest 0.1 mg. Digital images were used to determine snout-to-vent length and hind-limb length for each tadpole, using a computer image- processing program. For consistency, the left hind limb of each tadpole was measured. Hind-limb length was normalized by dividing by snout-to-vent length. Any tadpoles beyond Stage 60 by Day 21 were excluded from analyses of growth.

Histopathology: On Day 21, the tadpoles were fixed in Davidson's solution for at least 48 hours, rinsed with 70% ethanol, and placed in neutral buffered formalin. When possible, stage-matched tadpoles (5 from each replicate test chamber) were selected for histopathological processing and evaluation based on the median developmental stage of the negative controls. When there were fewer than five tadpoles at that stage, where available in a replicate, additional tadpoles were randomly selected from the developmental stages just above or below the median control developmental stage.

Histomorphologic parameters assessed included relative increases or decreases in the overall size of the thyroid glands, changes in follicular epithelial cell numbers or height, and alterations in colloid consistency. When appropriate, a scoring system to indicate the severity of these changes was used (Grade 0 = unremarkable, Grade 1 = mild, Grade 2 = moderate, and Grade 3 = severe).

Analytical measurements:

Water samples were collected from each replicate test chamber on Days 0, 7, 14, and 21 to measure concentrations of the test substance by means of HPLC. The limit of quantification (LOQ) was 0.100 mg a.s./L. Additional water samples were collected as needed during the test when previous results were questionable, or when there were interruptions in test substance delivery.

Statistical analysis:

Analyses were performed on survival, developmental stage, body weight, snout-vent length (SVL), normalized hind-limb lengths (HLL), and incidence and severity of thyroid abnormalities. Unless otherwise noted, the unit of statistical analysis was the replicate test chamber. If necessary, endpoints were analyzed using two complementary statistical approaches.

For growth parameters, endpoints were first evaluated for monotonicity. Since responses for these endpoints appeared to be monotonic, a step-down Jonckheere-Terpstra trend test was used to determine possible concentration responsive trends among the treatment groups. Body weight and SVL data also were analysed by performing pair-wise comparisons using Dunnett's multiple comparison test to further evaluate if those treatment groups differed statistically from the control group.

Data for endpoints analyzed by Dunnett's test were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Levene's test ($\alpha = 0.01$).

Survival was analyzed using Fisher's Exact test, and histopathology severity scores of individuals were analyzed using step-down Jonckheere-Terpstra trend tests only. Statistical tests used to evaluate treatment effects were performed at confidence level of $\alpha = 0.05$.

RESULTS

Measured concentrations of the pretest samples ranged from approximately 57 to 107% of nominal concentrations. Arithmetic mean measured concentrations are 81, 99, 108, 100 and 90% for the 0.16, 0.80, 4, 20, and 100 mg a.s./L treatment groups.

Table B.9.1.5-1: Summary of Treatment Concentrations in the Amphibian Metamorphosis Assay with Glyphosate

Nominal (mg a.s./L)	Initial measured (mg a.s./L)	Day 7	Day 14	Day 21	Mean measured (mg a.s./L)	Mean measured (% of nominal)	Mean CV (%)
Control	<LOQ	< LOQ	< LOQ	< LOQ	<LOQ	<LOQ	-
0.16*	0.185	0.156	<LOQ (d16: 0.102)*	0.134	0.13	81	41
0.80	0.834	0.790	0.736	0.793	0.79	99	5.2
4.0	<LOQ (d1: 4.15**	4.32	4.61	4.28	4.3	108	4.4
20	20.7	18.3	21.2	19.1	20	100	6.8
100	106	98.4	106	48.4***	90	90	31

* Day 14 samples were <LOQ due to a diluter malfunction. Day 16 samples confirmed that concentrations were returning to nominal once the diluter were repaired. Values of 50% of LOQ were used for Day 14 to calculate the mean measured concentration.

** Day 0 samples were <LOQ due to a sampling or processing error, confirmed by reanalysis of the samples. Additional samples analyzed on Day 1 confirmed the correct concentrations. The Day 0 samples were excluded from calculation of the mean measured concentration.

*** Results confirmed by analysis of the backup samples.

Mortality, Clinical Signs: There were no treatment-related effects on survival during the 21-day test. Mean percent survival to Day 7 was 100% in all treatment groups including control, except at 20 and

90 mg a.s./L, where mean survival was 98.8%. Mean percent survival to Day 21 was 98.8, 100, 100, 100, 97.5 and 98.8% in the 0, 0.13, 0.79, 4.3, 20, and 90 mg a.s./L treatment groups, respectively.

Control and treatment tadpoles generally appeared normal and healthy throughout the test. Beginning on Day 2 and continuing until test termination, tail curvature was observed in control and treatment tadpoles. By test termination, tail curvature was observed in more than 50% of all treatment groups, including the negative control. The tail curvature was considered to be neither treatment-related or a thyroid-related effect, but rather a dietary effect.

Developmental stage: No treatment-related effects on the median developmental stage were observed on Day 7 or Day 21. The median developmental stage of the tadpoles on Days 7 and 21 were 53 and 57, respectively, in all treatment groups including control. No observations of asynchronous development were noted by the authors. However, see further analysis below by the former RMS.

The developmental stages during the study are also presented in detail in the following table.

Table B.9.1.5-2: Distribution of developmental stages of African Clawed Frog tadpoles exposed to glyphosate for 21 days

Number of Tadpoles by Developmental Stage and Mean Measured Concentration (mg a.i./L)						
Developmental stage[1]	**Negative control (n=59)**	**0.13 (n=60)**	**0.79 (n=60)**	**4.3 (n=60)**	**20 (n=57)**	**90 (n=59)**
55	11	8	9	2	6	2
56	9	13	14	13	12	14
57	35	27	29	42	30	31
58	0	2	0	1	1	4
59	2	9	5	1	7	5
60	2	1	3	1	1	1
61	0	0	0	0	0	1
62	0	0	0	0	0	1
Median stage	57	57	57	57	57	57

1 The developmental stage was determined using a dissection microscope, and was based on the developmental stages described by Nieuwkoop and Faber (NF).

Tadpole Growth:

Hind-limb Length

No treatment-related effects on absolute or normalized hind-limb lengths were apparent on Days 7 or 21 (see table below).

Table B.9.1.5-3: Larval Development in African Clawed Frog (*Xenopus laevis*) – Hind- Limb Length. Values represent mean of four replicates

Treatment (mg a.s./L)	**Day 7**			**Day 14**		
	Mean (mm)	**SD**	**HLL:SVL**	**Mean (mm)**	**SD**	**HLL:SVL**
Control	2.08	0.10	0.13	7.65	0.68	0.33
0.13	2.10	0.08	0.13	8.48 (11%)	0.22	0.36
0.79	2.15	0.17	0.13	7.78 (1.7%)	0.43	0.33
4.3	1.75	0.21	0.11	8.20 (7.2%)	0.50	0.34
20	2.08	0.10	0.13	8.00 (4.6%)	0.69	0.34
90	2.10	0.14	0.13	8.25 (7.8%)	0.79	0.33

Snout-to-Vent Length (SVL) and Body Weight

Mean SVL was not significantly affected by glyphosate acid treatment on Day 7 (see table below). On Day 21, SVL was significantly increased ($p < 0.05$) compared to control in the 4.3, 20 and 90 mg a.s./L treatment groups by 5.2%, 2.5% and 6.7%, respectively, although this difference was not significant when normalized for hind-limb length. There was a significant increase (17%) in Day 21 body weight at 90 mg a.s./L. It was proposed however, that growth should not be solely relied upon to determine

thyroid toxicity. Rather, growth, in conjunction with developmental stage and thyroid histopathology, should be used to determine thyroid activity.

Table B.9.1.5-4: Larval Growth in African Clawed Frog (*Xenopus laevis*)

Treatment (mg a.s./L)	Snout-Vent Length (SVL)				Body weight (wet weight)			
	Day 7		Day 21		Day 7		Day 21	
	Mean (mm)	SD	Mean (mm)	SD	Mean (g)	SD	Mean (g)	SD
Control	15.8	0.98	23.2	0.43	0.267	0.040	0.864	0.038
0.13	15.9	0.38	23.6	0.66	0.273	0.021	0.925	0.100
0.79	16.1	0.75	23.5	0.83	0.288	0.031	0.907	0.078
4.3	16.1	0.80	24.4*	0.15	0.290	0.042	0.973	0.037
20	16.1	0.34	23.8*	0.45	0.282	0.022	0.920	0.056
90	16.1	0.99	24.8*	0.38	0.300	0.048	1.01*	0.060

*statistically significant $p<0.05$

Histopathology: Observations and severity of thyroid atrophy and hypertrophy, and follicular cell hypertrophy and hyperplasia are presented in the table below. There appears to be an increased incidence of mild thyroid gland hypertrophy in the highest treatment concentration, however, it was proposed that since the same incidence was observed at the lowest treatment concentration the effect was not concentration responsive. Similar findings were observed for follicular cell hypertrophy. The pathology analysis concluded that there were no treatment related changes in the thyroid glands of exposed tadpoles when compared to organisms in the negative control.

Table B.9.1.5-5: Gross Histopathology of the Thyroid Gland in African Clawed Frog (*Xenopus laevis*)

Treatment (mg a.s./L)	Severity*	Thyroid gland hypertrophy		Thyroid gland atrophy		Follicular cell hyperplasia		Follicular cell atrophy	
		n	incidence	n	incidence	n	incidence	n	incidence
Control	0	20	17	20	19	20	17	20	17
	1	20	3	20	1	20	1	20	2
	2	20	0	20	0	20	2	20	1
	3	20	0	20	0	20	0	20	0
0.13	0	20	14	20	17	20	14	20	16
	1	20	4	20	3	20	4	20	2
	2	20	2	20	0	20	1	20	2
	3	20	0	20	0	20	1	20	0
0.79	0	20	17	20	17	20	13	20	17
	1	20	1	20	2	20	3	20	3
	2	20	2	20	1	20	3	20	0
	3	20	0	20	0	20	1	20	0
4.3	0	20	18	20	18	20	16	20	17
	1	20	1	20	2	20	2	20	3
	2	20	1	20	0	20	2	20	0
	3	20	0	20	0	20	0	20	0
20	0	20	18	20	19	20	18	20	15
	1	20	0	20	1	20	1	20	4
	2	20	2	20	0	20	1	20	1
	3	20	0	20	0	20	0	20	0
90	0	20	14	20	18	20	14	20	17
	1	20	6	20	2	20	2	20	3
	2	20	0	20	0	20	4	20	0
	3	20	0	20	0	20	0	20	0

The test was regarded as valid, since the following criteria according to OECD 231 guideline (2009) were met:

- The dissolved oxygen concentration was at least 40% of the air-saturation value throughout the exposure period.
- Water temperature did not differ by more than 1ºC between test vessels at any one time during the exposure period, and were maintained within ±1ºC of the 22ºC temperature specified.
- There was at least 90% survival of control animals over the duration of the exposure period, and mortality in any one control replicate did not exceed two tadpoles.
- Test concentrations were consistent over the course of the study (i.e., contained at ≤20% CV over the 21-day test), except for low recoveries in the low treatment group on Day 14 and in the high treatment group on Day 21.
- The minimum median stage of the control tadpoles at the end of the test was at least 57.
- The 10^{th} and the 90^{th} percentiles of the developmental stage distribution did not differ by more than 4 stages.
- There were less than two non-control test concentrations with overt toxicity.
- There were less than two replicates across the test that were compromised.

CONCLUSIONS

Assessment and conclusion by applicant:

Arithmetic mean measured concentrations are 81, 99, 108, 100 and 90% for the 0.16, 0.80, 4, 20, and 100 mg a.s./L treatment groups. Therefore, results can be expressed as nominal concentrations.

There were no treatment related effects on survival, stage, or normalized hind-limb length during the 21 day test. Histopathologic analysis showed no treatment related changes in the thyroid glands of *Xenopus laevis* tadpoles when compared to negative control animals. There was a slight increase in wet weight in the 100 mg a.s./L treatment group and in snout-to-vent length in the 4.0 and 100 mg a.s./L treatment groups at the end of the 21-day test, however, this difference in snout-vent length was not significant when normalized with hind-limb length. Since there were no effects observed on normalized hind-limb length, stage, or thyroid histology, these increases are not indicative of a thyroid effect. Glyphosate acid was not found to interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis of African clawed frog tadpoles in this study.

The Amphibian metamorphosis assay (AMA) with the African clawed frog (*Xenopus laevis*) exposed to glyphosate acid is considered valid and the overall NOEC ≥100 mg a.s./L (arithmetic mean measured) can be used for ecotoxicological risk assessment.

Assessment and conclusion by RMS:

The study was performed according to OECD 231 and is considered valid and acceptable. It is proposed though that since the test concentrations dropped below 80% of the nominal values, the derived endpoints from the study are expressed as mean measured. We agree with former RMS calculation of the mean measured concentrations at the highest treatment level (85% of nominal).

We also agree with the former RMS that the overall interpretation of the test results by the performing laboratory and by the applicant is considered plausible. Specific comments and further analysis made by the former RMS are presented below.

There seems to be a possible higher incidence of thyroid gland hypertrophy at the highest dose. However, probably the replication in the test is not sufficient to statistically confirm this as a treatment related effect. The findings should also be evaluated in the context of other relevant information from the toxicology section.

According to the new ED-guidance (Echa/EFSA 2018), treatment levels should be selected in order to ensure that the MTC is covered. In this case, the dose selection was based on a range-finding study with doses of 6.25, 12.5, 25, 50 and 100 mg a.i./L for 14 days, where only one incidental mortality at 50 mg a.i./L was observed. It should also be noted that the nominal concentrations decreased during the study (see evaluation by former RMS below). Since no toxicological effects from the treatments were confirmed, it is indicated that the MTC was not reached. On the other hand, OECD 231 recommends that the highest tested dose should be "*set by the solubility limit of the test substance; the maximum tolerated concentration (MTC) for acutely toxic chemicals; or 100 mg/L, whichever is lowest."* It is noted that the range finding test was performed up to the limit dose of 100 mg/L and the final test was performed at 90 mg/L (measured), or 100 mg/L (nominal), since the range finder did not indicate significant acute toxicity below that level and the solubility of glyphosate is much higher
(10.1 g/L). As a result, since the test was performed at the limit dose suggested by OECD 231, the study is considered to be valid.

It is agreed with the former RMS that the results of this study do not indicate an endocrine activity via thyroid pathways in *Xenopus*.

Specific comments by the former RMS (Addendum 2_Potential ED properties_rev 2_2017-07-04)

In the context of the overall test performance and results, the malfunction of the flow-through system delivering temporarily incorrect test concentrations in some of the test treatments does in the opinion of the RMS not to constrain the validity of the study regarding its specific aims.

However, it should be noted that the glyphosate concentrations in the test were unstable over the study in the lowest and highest tested treatments. Here, the measured glyphosate concentrations at the end of the study were in the range of 48% of the nominal test concentration of 100 mg a.s./L (mean measured at day 0 = 106 ; at day 7= 98.4; at day 14= 105.5; and at day 21 = 48.4 mg a.s./L). Considering the measurements till day 14, a mean measured concentration of 103.3 mg a.s./L was detected. Taking this value twice to account for the two week exposure period and averaging over the three weeks test duration with the data of day 21, a mean measured concentration of 85 mg glyphosate/L can be calculated. Since there were no measurements between day 14 and day 21, it cannot be excluded that the reduced exposure of tadpoles to glyphosate in this treatment was prolonged over some days towards the study end.

The pH of the test solutions was lower in treatments with higher glyphosate concentrations compared to the control vessels. Especially in the highest glyphosate treatment, the pH of the test solution was more than one pH unit lower than in the control. While water hardness and specific conductance were similar, water alkalinity was accordingly reduced in the highest glyphosate treatment compared to control vessels (175 ± 4 vs. 148 ± 3 mg $CaCO_3$ * L-1 in control and highest glyphosate treatment, respectively).

As additional information to the evaluation by the performing laboratory and by the notifier, the determined weight of the tadpoles in the test was further analyzed by (the former) RMS. It is correct to state that at test end the higher tadpole weight at the highest tested glyphosate concentration was statistically significant different compared to the tadpoles in the control treatment. It seems, though, that a relationship exists between tadpole weight at day 7 and weight at day 21 (please refer the figure below). However, no clear treatment dependency could be detected. In the opinion of the (former) RMS, the random attribution of tadpoles to the different treatments at test start was possibly not optimal.

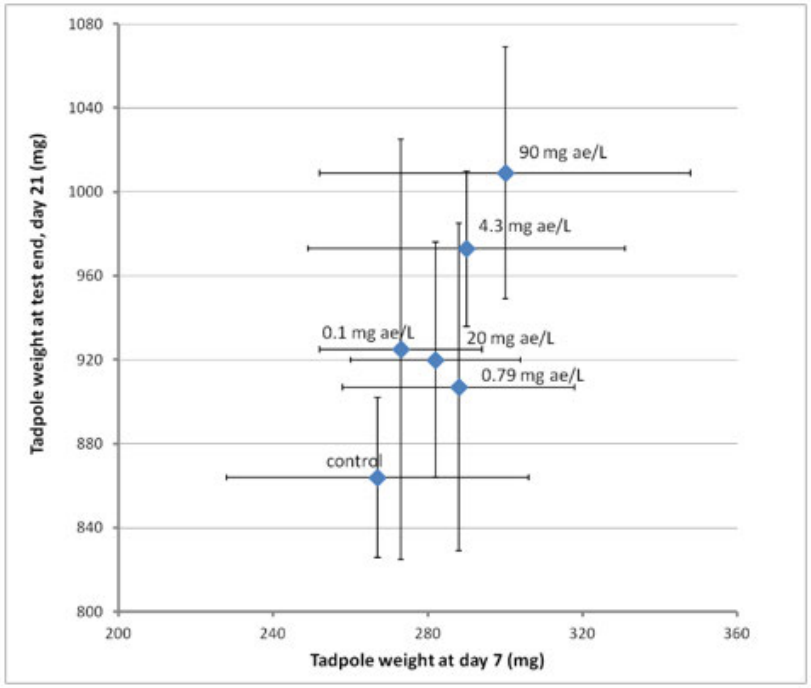

Figure B.9.1.5-1: Weight (mg) of *Xenopus laevis* tadpoles exposed to glyphosate according to OECD 231 standard protocol. Displayed is the weight at test end (day 21) related to weight at day 7 of exposure. Data from [redacted] 2012a, evaluation former RMS (Addendum 2017)

Regarding developmental stages reached by the tadpoles till study end, the performing laboratory and by the applicant summarize the results by correctly stating that the median tadpole stage at test end was for all treatments stage 57 according to Nieuwkoop and Faber (NF).

When looking at the distribution of the treatment tadpole cohorts to the different stages, though, there is a slight shift towards later developmental stages reached by tadpoles exposed to higher glyphosate concentrations. In the control treatments, there are more tadpoles that at day 21 are in earlier developmental stages (e.g. NF 55) compared to the treatments with higher glyphosate concentrations (see figures below). The tadpole group exposed to the highest glyphosate concentration tested was the only one with single animals reaching stage NF 61 and 62. The differences between stage distribution in the cohorts of the control treatment are not statistically significantly different from the highest glyphosate treatment if tested with two sided Mann-Whitney-U test ($p = 0.052$).

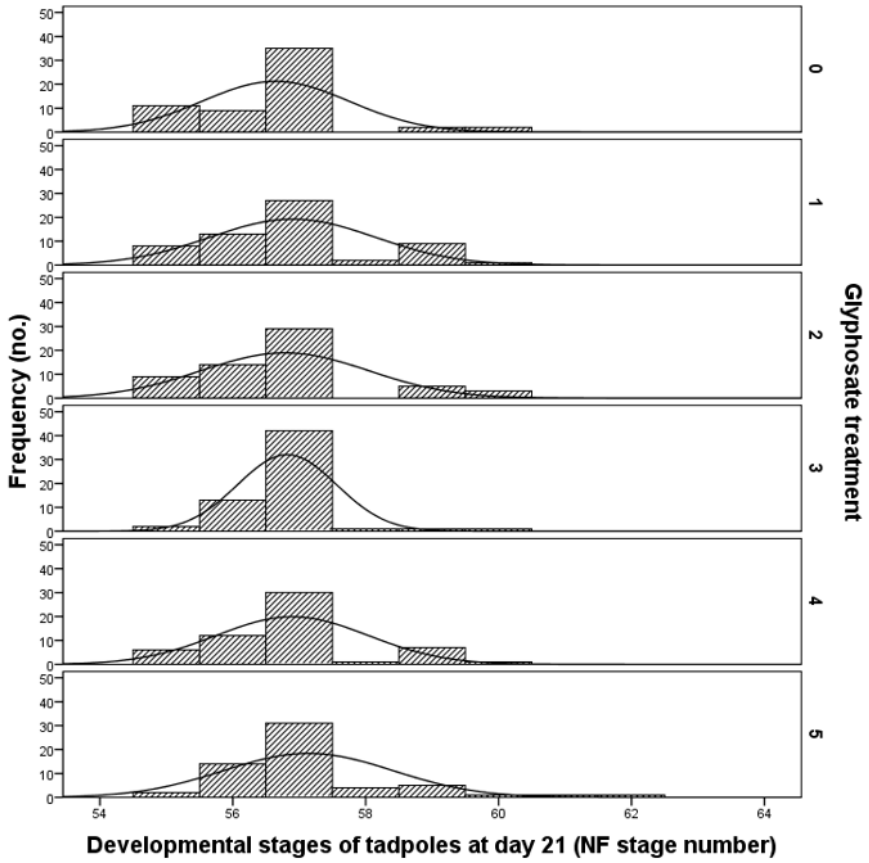

Figure B.9.1.5-2: Developmental stages of *Xenopus laevis* tadpoles (according to Nieuwkoop and Faber, NF) exposed to glyphosate in different treatments. T0: control, T1 = 0.13; T2 = 0.79; T3 = 4.3; T4 = 20 and T5 = 90 mg acid equivalent/L. Data from [redacted] 2012a, evaluation former RMS (Addendum 2017).

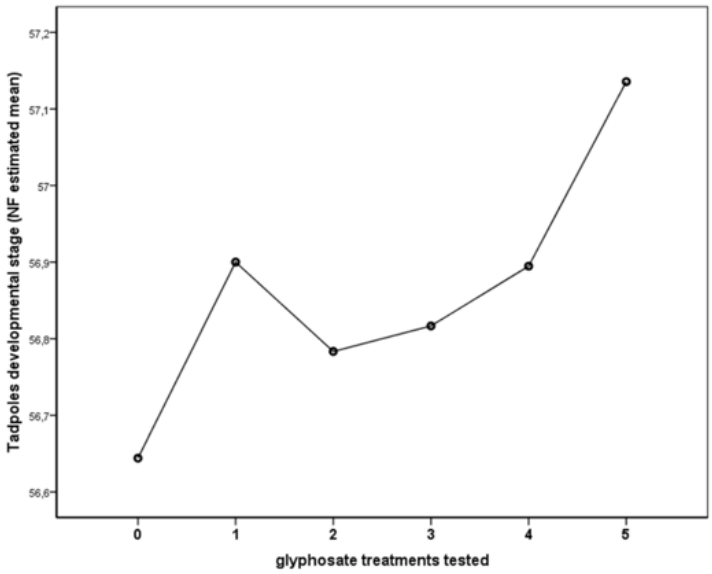

Figure B.9.1.5-3: Line plots of mean tadpoles developmental stage at day 21 exposed to glyphosate in different treatments. Data from this report ([redacted] 2012a), evaluation former RMS

Summarizing the above, there are significant effects of glyphosate exposure on the endpoints tadpole growth and tadpole snout-to-vent length in the highest tested glyphosate treatment (nominal 90 mg a.e /L) when compared to the control treatment. In the two tested treatments below 90 mg a.e./L -which were 4.3 and 20 mg a.e./L – the snout-to-vent length showed an increasing trend compared to the control

treatment (Jonckheere-Terpstra trend test; p ≤0.05). However, while in the 4.3 mg a.e./L the tadpole snout-to-vent length was statistically significant different from the control (Dunnet test p ≤0.05), the differences in tadpole length between the 20 mg a.e./L treatment and the control was not statistically significant. The reported differences could not be observed when snout-to-vent length was normalized to hind-limb length. See also summary table with HLL and HLL:SVL data in the results section above.

The incidence in diagnostic observation in the gross histopathology of thyroid gland of African Clawed Frog (*Xenopus laevis*) at test end showed no treatment related effects on the observed endpoints changes in thyroid gland size, follicle size and asymmetry. Thyroid follicular epithelium showed also no sign of hyperplasia or hypertrophy due to increased glyphosate concentrations.

The former RMS concluded that the results of this assay do not point at disturbed thyroidal activity due to glyphosate exposure of the tested species.

A feature that is not requested by Guidance OECD 231 is the parallel testing of a positive reference substance along with the compound to be screened for endocrine activity. In the view of the former RMS, though, the testing of a positive control would have been welcomed as great added value for the interpretation of the test results, requiring however additional test animals. Even if not required by the guidance, the sensitivity of the organisms in the assay remains unknown.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

Data point	CA 8.2.1/001
Report author	[redacted]
Report year	2003
Report title	MON 78623: A 96-hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
Report No	139A-310C
Document No	-
Guidelines followed in study	OECD Guideline 203 OPPTS 850.1075
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation compared with OECD 203:* *Major:* *- none* *Minor:* *The temperature was lower than recommended (12.2 – 12.7 °C instead of the recommended 13 – 17 °C), since it has been found to be an acceptable temperature to maintain healthy rainbow trout.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

The toxicity of glyphosate potassium (K) salt on rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour static (without media renewal) toxicity test conducted at nominal test concentrations of 156, 313, 625, 1250 and 2500 glyphosate K-salt/L, corresponding to 74.4, 149, 298, 596 and 1193 mg glyphosate acid/L (mg a.e./L). A negative control group (dilution water only) was also prepared.

Duplicate vessels were prepared for the control and each test item level, with 10 fish added to each vessel.

Observations for sub-lethal effects and mortality were performed at 4, 24, 48, 72 and 96 hours after the start of the test (fish addition). The pH-value and oxygen saturation of the test solutions were measured at test initiation and at daily intervals. Temperature was measured at test initiation and termination. Samples of test media were taken at the start (before fish addition), and after 48 and 96 hours for the analysis of glyphosate K salt using an HPLC method of analysis. Overall mean measured glyphosate K-salt concentrations were 159, 329, 646, 1302 and 2573 mg a.s./L. Glyphosate K-salt was not detected in the control group. Measured concentrations ranged from 99.8 to 109% of nominal concentrations. Toxicity evaluations were based on nominal concentrations.

There was no mortality in the control, 156, 313 and 625 mg a.s./L treatment groups. In the 1250 and 2573 mg a.s./L treatment groups, there was 5 and 15%, respectively, with significant sub-lethal effects (including erratic swimming, and loss of equilibrium) observed in the 625, 1250 and 2500 mg a.s./L treatment groups within 15 minutes of fish addition. Test media pH was negatively correlated with test concentration. All validity criteria according to the guideline OECD 203 were fulfilled.

The 96 hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate K-salt was determined to be > 2500 mg a.s./L, equivalent to >1193 mg a.e./L. The 96 hour NOEC was determined to be 313 mg a.s./L, equivalent to 149 mg a.e./L.

I. MATERIALS AND METHODS

1. Test material:

Test item:	MON 78623 (Glyphosate K-salt)
Description:	yellow liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	47.7%
Vehicle	Vehicle: dechlorinated and filtered tap water

2. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juvenile
Size (mean standard length):	43 mm (38 – 56 mm)
Weight (mean wet weight):	0.94 g (0.59 – 1.3 g)
Loading:	0.47 g fish/L
Source:	[redacted]
Acclimation period:	5 weeks prior to the test initiation

3. Environmental conditions:

Temperature:	12.2 – 12.7°C
Photoperiod:	16 h light, with a 30 min transition period
pH:	Control (start – 96 h): 8.2 – 8.0 156 mg/L (start – 96 h): 7.5 – 8.1 313 mg/L (start – 96 h): 7.1 – 8.0 625 mg/L (start – 96 h): 6.7 – 7.9 1250 mg/L(start – 96 h): 6.2 – 7.1 2500 mg/L (start – 96 h): 5.7 – 5.8

Dissolved oxygen: ≥ 7.3 mg/L (≥ 67% saturation)
Conductivity: 280 µS/cm
Hardness: 144 mg $CaCO_3$/L.
Alkalinity: 184 mg $CaCO_3$/L

4. Dates of experimental work: 21th February to 25th February 2003

B. STUDY DESIGN

Experimental treatments: A definitive toxicity test was performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg a.s./L (mean measured: 159, 329, 646, 1302 and 2573 mg a.s./L) in a static test setup, based on the results of a range finding test,. A negative control group (dilution water only) was prepared in parallel. Duplicate vessels (38 L glass vessels containing 20-L control water or test medium) were prepared for the control and treatment groups, each containing ten fish (20 fish per treatment).

Observations: Observations for sub-lethal effects and mortality were performed at 4, 24, 48, 72 and 96 hours after test initiation (fish addition). The pH-value and oxygen saturation of the test solutions were measured at test initiation and on each observation date. Temperature was measured at test initiation and termination. Hardness, alkalinity and specific conductivity of the test water were measured at the start of the test only. Fish wet weights and total lengths were measured in the control. Samples of control or test media from all vessels was taken at 0 (before fish addition) 48 and 96 hours and analysed to determine the to measure glyphosate K salt concentration.

Statistical calculations: Since the mortality was < 50%, no statistical calculation of LC_{50} values was possible. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Chemical analyses were performed on samples of the test solutions to quantify glyphosate concentrations in the test solution. Measured concentrations were between 99.8 and 109% of nominal confirming the stability of the test substance in the test system. The ecotoxicological endpoints are based on the nominal concentrations of 156, 313, 625, 1250 and 2500 mg glyphosate K-salt /L. The limit of quantitation (LOQ) was 10.5 mg/L (5.0 mg a.e./L).

Table B.9.2.1-1: Analytical results

Nominal concentrations Glyphosate K-salt [mg a.s./L]	Mean measured concentration Glyphosate K-salt [mg a.s./L]	% of nominal	Mean measured concentration Glyphosate acid equivalent [mg a.e./L]
Control	< LOQ	-	-
156	159	102	74.4
313	329	105	149
625	646	103	298
1250	1302	104	596
2500	2573	103	1193

The 96 hour LC_{50} and NOEC values for rainbow trout (*Oncorhynchus mykiss*) exposure to glyphosate K-salt based on nominal concentrations are given below.

Table B.9.2.1-2: Endpoints

Endpoints	Expressed as Glyphosate K-salt [mg a.s./L]	Expressed as Glyphosate acid [mg a.e./L]
96 h LC_{50}	> 2500	1193
96 h NOEC	313	149

B. OBSERVATIONS

There was no mortality or sub-lethal effects in the negative control and at the mean measured concentrations of 156 and 313 mg glyphosate K salt/L. At 625, 1250 and 2500 mg glyphosate K-salt/L, 0, 5 and 15% mortality were observed respectively.

At the three highest test concentrations, sub-lethal effects were noted within 15 minutes after test initiation (including surfacing, laying on the bottom of test chamber, erratic swimming, loss of equilibrium).

The severity of effect generally increased with increasing concentration, which correlated to the concentration-responsive decrease in pH. The pH at 0 h decreased from 8.2 for the controls to 5.7 at the highest test concentration. All surviving fish in 625 and 1250 mg a.s./L appeared normal by 24 h and appeared normal for the remainder of the test. Effects were still evident in three of the 17 surviving fish in 2500 mg test item/L at test termination. The pH remained below 6 in the highest test concentration throughout the test.

The biological results achieved during the fish acute toxicity test are presented below:

Table B.9.2.1-3: Lethal effects of glyphosate K-salt to rainbow trout (*Oncorhynchus mykiss*)

Glyphosate K-salt [mg a.s./L]	Glyphosate acid [mg a.e./L]	Number of dead fish / number of fish with intoxication symptoms and observed symptoms					
		0 h	4 h	24 h	48 h	72 h	96 h
Control		0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
156	**74.4**	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
313	**149**	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
625	**298**	0 / 20	0 / 11 A	0 / 0	0 / 0	0 / 0	0 / 0
1250	**596**	0 / 3 R / 17 E,N	1 / 17 A / 2R	1 / 0	1 / 0	1 / 0	1 / 0
2500	**1193**	0 / 8 R / 12 E,N	0 / 7 R / 13 A,E,N	0 / 6 R / 4 A / 4 E, 2 N	3 / 0	3 / 3 R / 1 C	3 / 3 R

A = surfacing; R= laying at bottom of test chamber; E = erratic swimming, N = loss of equilibrium

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

Assessment and conclusion

Assessment and conclusion by applicant:
The 96 hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to the glyphosate K-salt was determined to be > 2500 mg a.s./L (nominal), corresponding to >1193 mg a.e./L. The 96 hour NOEC was determined to be 313 mg a.s./L, corresponding to 149 mg a.e./L.

This study is considered valid and the acute LC_{50} for rainbow trout exposed to glyphosate K-salt was determined >1193 mg a.e./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
This study is valid.

It was reported as minor deviations that "The temperature was lower than recommended (12.2 – 12.7 °C instead of the recommended 13 – 17 °C), since it has been found to be an acceptable temperature to maintain healthy rainbow trout." According to the current OECD 203 guideline (2019) there is no deviation from the recommended temperature when compared to the preferred range (10 -14°C).

Acute LC50 for rainbow trout exposed to glyphosate K-salt >1193 mg a.e./L (nominal).
NOEC = 149 mg a.e./L (nominal)

Data point:	CA 8.2.1/002
Report author	[redacted]
Report year	1995
Report title	Glyphosate acid: Acute Toxicity to rainbow trout (*Oncorhynchus mykiss*)
Report No	[redacted]0503/D
Document No	
Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019): None except the levels of pH which declined with increasing concentration of the test item (below the recommended range at the four highest doses)*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid with restrictions (pH issues at 180 mg/L)

Executive Summary

The acute effects of glyphosate acid to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-hour static toxicity test conducted at nominal test concentrations of 32, 56, 100, 180, 320 and 560 mg glyphosate acid/L. A dilution water control was also included in the test. Ten fish were exposed in the control and in each treatment. All fish were observed at daily intervals over the 96 hour study duration, with mortality and sub-lethal signs of toxicity recorded.

Dissolved oxygen, pH and temperature were measured daily in each test vessel. Samples of control and test media were analysed for glyphosate acid at 0 hours (before fish addition) and after 48 and 96 hours. Glyphosate acid was not detected in the control group. The overall mean measured concentrations of glyphosate acid in the treatment groups ranged from 91 to 100% of nominal concentrations.

There were no fish mortalities or sublethal effects in the control group. At the 32, 56 and 100 mg a.s./L treatments, there were also no fish mortalities but there were transient sublethal effects including dark dicolouration and loss of balance, observed in the 56 and 100 mg a.s./L treatments. All fish in these three groups appeared normal at 96 hours, whilst in the 180, 320 and 560 mg a.s./L there was 100% mortality. All validity criteria according to the guideline OECD 203 were fulfilled.

The authors concluded that the 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid was determined to be 130 mg a.s./L (nominal) with a 95% confidence interval of 100 to 180 mg a.s./L. The 96-hour NOEC value was 32 mg a.s./L. This study is considered valid.

The RMS concluded that the study is valid with retsrictions because of pH issue at concentrations greater tha 100 mg/L. The 96-hour LC50 is considered to be greater than 100 mg glyphosate acid/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %

2.Vehicle of test material/media: dechlorinated and filtered tap water

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juvenile
Size:	Length: 40 – 71 mm (mean: 57 mm)
Body weight of the animals:	1.16 – 4.56 g/fish (mean: 2.68 g)
Loading:	0.89 g fish/L (10 fish per 30 litres of test medium)
Source:	[redacted]
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	32 days

4. Environmental conditions:

Temperature:	11.5 – 12.6°C
Photoperiod:	16 hours

pH:	Control (start – 96 h): 7.7 - 7.0 32 mg/L (start – 96 h): 6.4 – 6.2 56 mg/L (start – 96 h): 5.9 – 6.0 100 mg/L (start – 96 h): 4.7 – 5.1 180 mg/L(start – 24 h): 3.5 320 mg/L (start – 24 h): 3.0 560 mg/L (start – 24 h): 2.8 – 2.7
Dissolved oxygen:	6.2 – 10.4 mg O_2/L
Conductivity:	281 µS/cm³ in the dilution water
Hardness:	56.3 mg $CaCO_3$/L
5. Dates of experimental work:	September 11th to September 15th 1995

B. STUDY DESIGN

Experimental treatments: The toxicity test was performed at nominal concentrations of 32, 56, 100, 180, 320 and 560 mg a.s./L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions. A negative control (dilution water only) was also prepared. A single replicate vessel was prepared for the control and at each treatment level, each containing ten fish (added to 40 L glass aquariums containing 30 L test medium).

Observations: Fish in all vessels were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. At test termination, the ten fish from the dilution water control were weighed and measured. Analytical measurements were performed by HPLC analysis at test initiation and after 48 and 96 hours.

Analytical procedures: Samples were taken from the centre of the test solutions. Glyphosate acid concentrations in the test solutions were determined at 0, 48 and 96 hours by high performance liquid chromatography method using a fluorescence detector. The samples were quantified against standards of glyphosate acid. Prior to analysis, samples and standards were derivatised using flourenylmethyl chlorformate, to prepare a fluorescing derivate.

Statistical calculations: The LC_{50} values and their 95% confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The mean measured concentrations of glyphosate acid ranged from 91 to 100%. As the measured concentrations of glyphosate were between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item. The limit of detection was 0.004 mg/L.

Table B.9.2.1-4: Analytical results

Nominal concentration Glyphosate acid [mg a.s./L]	Measured concentration Glyphosate acid at 0 hours [mg a.s./L]	Measured concentration Glyphosate acid at 48 hours [mg a.s./L]	Measured concentration Glyphosate acid at 96 hours [mg a.s./L]	% of nominal
Dilution water control	< 0.004	< 0.004	< 0.004	-
32	29	29	29	91
56	54*	54*	55*	96
100	94	91	94	93
180	180	170	-	100
320	320	320	-	100
560	550	540	-	98

- Not sampled, 100% mortality on previous sampling occasion
* mean of triplicate analysis

The 96 hour LC_{50} and NOEC values are presented below.

Table B.9.2.1-5: Endpoints

Endpoints	Glyphosate acid [mg a.s./L]
LC_{50} (95% C.L.) (96 h)	130 (100 – 180)
NOEC (96 h)	32

B. OBSERVATIONS

Until 100 mg a.s./L no mortality occurred, but all fish died at the test concentrations of 180 mg a.s./L and higher. Transient sublethal effects of dark discolouration and loss of balance were observed at 56 and 100 mg a.s./L respectively. All surviving fish in the study appeared normal at the end of test.

All measured water quality parameters were within the specifications recommended by the OECD 203 test guideline, except pH, where the levels of pH declined with increasing concentration of the test item. At 180 mg a.s./L, the pH was 3.5 and lower.

The biological observations recorded during the test are presented below.

Table B.9.2.1-6: Effects of glyphosate acid to rainbow trout

Nominal concentration of glyphosate acid [mg a.s./L]	Number of dead fish / number of fish with intoxication symptoms [1] and observed symptoms			
	24 h	48 h	72 h	96 h

Control	0 / 0	0 / 0	0 / 0	0 / 0
32	0 / 0	0 / 0	0 / 0	0 / 0
56	0 / 0	0 / 0 DC (between 11-30% test population)	0 / 0 DC (between 11-30% test population)	0 / 0
100	0 / 0 DC	0 / 0 DC, LB (between 11-30% test population)	0 / 0	0 / 0
180	* / *	* / *	* / *	* / *
320	* / *	* / *	* / *	* / *
560	* / *	* / *	* / *	* / *

[1] Dead fish are added to the sum of fish with symptoms
* / * All fish dead
DC: Dark colouration; LB: Loss of balance

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥60 % of air saturation and constant exposure conditions have been maintained.

Assessment and conclusion

Assessment and conclusion by applicant:
The 96 hour LC_{50} value for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid was calculated to be 130 mg a.s./L (nominal) with 95% confidence interval of 100 to 180 mg a.s./L. The NOEC after 96 h was 32 mg a.s./L.

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to glyphosate acid was determined to be 130 mg a.s./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
The pH values at the three highest tested concentrations (180, 320, 560 mg glyphosate acid/L) were far below the recommended range (recommended pH 6-8.5). The actual pH values were of 3.45 at 180 mg/L, 3 at 320 mg/L and 2.74 at 560 mg/L. This deviation was negatively correlated with increasing concentrations of glyphosate indicating that this pH decrease was due to the test item (intrinsic biochemical characteristic of glyphosate acid).

The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options.
In Schweizer, M. et al (2019) (CA 8.2.2.1/006 see addendum of B.9 (AS) on general literature review), zebrafish (*Danio rerio*) embryos acutely exposed to glyphosate at concentrations between 1.69 and 1690.7 mg glyphosate/L for 96 hours post fertilization in buffered and unbuffered situation. A particular attention was paid on pH from 3 to 4. The study demonstrates that the severe effects detected seemed to be mainly caused by a low (glyphosate induced) pH.
Overall, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the

pH should always be in a range required to maintain the health of the organisms tested (here 6.5-8.5). Therefore, as pH are below is 4 for test concentration equal or greater than 180mg/L, it can not be excluded that the high mortality observed at these concentrations was due to the acidity of the test solutions caused by high concentrations of glyphosate acid.

At 56 and 100 mg/L, pH was of 5.86 and 4.71 respectively (and no mortality was observed). This pH is slightly below the recommended value.
Recommended length was slightly exceeded for some fish (max 71 mm > recommended 60 mm) but the overall mean was 57 mm. The loading also slightly exceeded the recommended value (0.89 g fish/L > recommended 0.8 g/L). These 2 deviations are considered minor and not likely to affect the outcome of the study.
This study is valid according to validity criteria.
Acute LC50 value for rainbow trout exposed to glyphosate acid > 100 mg a.s./L (nominal).
NOEC after 96 h = 32 mg a.s./L.

Data point	CA 8.2.1/003
Report author	[redacted]
Report year	1995
Report title	The acute toxicity of glyphosate to Rainbow trout (*Oncorhynchus mykiss*)
Report No	710/21
Document No	-
Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Toxicity of technical glyphosate (purity >94 %) to aquatic organisms (*Oncorhynchus mykiss*) in a 96 hours static test
Short description of results:	LC_{50} >100 mg a.e./L and NOEC >100 mg a.e./L
Reasons why the study is not considered relevant/reliable or not considered as key study	The full study report is not available to the applicant. However these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR (2015).
Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS:
RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was requested to the BVL but could not be provided to RMS.

For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical. The endpoint measured in this study is not critical and therefore its absence has no consequence on the risk assessment.

Data point:	CA 8.2.1/004
Report author	[redacted]
Report year	1993
Report title	Acute Toxicity Testing in Fish Test Article: 'Glyphosate isopropylamine salt'
Report No	80-91-2328-03-93
Document No	
Guidelines followed in study	OECD Guideline 203; EEC Directive 92/69
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *None*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

The effects of glyphosate isopropylamine salt on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. The toxicity test was performed using nominal concentrations of 107, 235, 517, 1136 and 2500 mg test item/L, corresponding to 65.9, 145, 318, 700 and 1540 mg glyphosate isopropylamine salt/L (mg a.s./L) or 48.8, 107, 236, 519 and 1141 mg glyphosate/L (mg a.e./L). Further a dechlorinated and deionised tap water control was tested. Ten fish were exposed to each treatment level.

Mortality was recorded after 2-4, 24, 48, 72 and 96 hours after the start of the test. Records on visible abnormalities were equally made. At termination of the test, all animals were weighed and measured.

At the nominal concentration of 1136 and 2500 mg test item/L, after 24 h of exposure the fish showed reduced activity and a tendency of staying at the bottom of the test vessels. In comparison to the control group, no obvious abnormal effects were seen at or below the concentration of 517 mg test item/L. All validity criteria according to the guideline OECD 203 were fulfilled.

In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for rainbow trout exposed to glyphosate isopropylamine salt was determined to be 2192 mg test item/L, corresponding to 1350 mg a.s./L or 1001 mg a.e./L (nominal). The NOEC was determined to be 517 mg test item/L, corresponding to 318 mg a.s./L or 236 mg a.e./L (nominal). This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	Viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C

2. Vehicle of test material/media: dechlorinated and deionised tap water

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Not stated
Size:	Length: 6.70 cm (mean of 10 representative individuals)
Loading:	10 L for 5 fish
Source:	Commercial supplier [redacted]
Acclimation period:	≥ 48 h in a 250 L glass aquarium under general test conditions
Body weight of the animals:	1.92 g (mean body weight of 100 individuals)

4. Environmental conditions:

Temperature:	14.5 – 16.3°C
Photoperiod:	16 hours light / 8 hours dark, 600 - 800 lux
pH:	5.7 – 8.4
Dissolved oxygen:	8.2 – 10.2 mg O_2/L)
Conductivity:	Not stated
Hardness:	14° dH (1dH= 10 mg CaO/L)

5. Dates of experimental work: 24th August to 04th September 1993

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, definitive toxicity test was performed using nominal concentrations of 107, 235, 517, 1136 and 2500 mg test item/L in a static test setup. In addition a control group was exposed to dechlorinated and deionised tap water only. There were two vessels per treatment, each containing five fish (12 L glass containers containing 10 L test medium)

Observations: Assessment of effects and mortality of test fish after 2-4, 24, 48, 72 and 96 hours was conducted. Temperature, pH-value and oxygen saturation (% air saturation value [% ASV]) of the test solutions were measured on a daily basis. Hardness of the test water was measured at the start of the test.

Mortality was recorded on each observation date. Records on visible abnormalities were equally made. At start and termination of the test, all animals were weighed and measured.

Analytical control measurements of the actual concentrations of the test item were performed by mean of HPLC analysis. Glyphosate isopropylamine salt levels were determined based on the concentrations of glyphosate. Three representative concentrations (107, 517 and 2500 mg test item/L, corresponding to 65.9, 318 and 1540 mg a.s./L or 48.8, 236 and 1141 mg a.e./L) were analysed at 24 h intervals.

Statistical calculations: 24 h, 48 h, and 72 h LC_{50} values were determined directly from the raw data. The 96 h LC_{50} value was calculated by Probit analysis according to Finney (1971).

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values are given below based on nominal concentrations.

Table B.9.2.1-7: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50} (95% C.L.))	2192 (1501-19088)	1350	1001
NOEC	517	318	236
LOEC	1136	700	519

Analytical data: Analytical control measurements were performed on three representative concentration levels of glyphosate isopropylamine salt, at 107 mg test item/L, corresponding to 48.8 mg a.e./L, 517 mg test item/L, corresponding to 236 mg a.e./L and at the highest concentration tested, 2500 mg test item/L, corresponding to 1141 mg a.e./L. Before introduction of the fish 99.2 %, 102.7% and 95.1% of glyphosate were recovered at 107, 517 and 2500 mg test item/L, respectively. In the aged test media 95.2%, 90.3% and 85.1% of the nominal concentration were recovered. Consequently, during the test period of 96 hours the fish were exposed to a mean concentration of 90.2% (average for test concentrations of 107, 517 and 2500 mg test item/L, respectively) of nominal concentration.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.1-8: Analytical results

Nominal concentration of test item [mg/L]	Nominal concentration of glyphosate [mg a.e./L]	Time (hours)	Measured concentration of glyphosate [mg a.e./L]	% of nominal
107	48.8	0	48.44	99.2
		48	47.79	97.9
		96	46.50	95.2
517	236	0	242.45	102.7
		48	215.31	91.2
		96	213.09	90.3
2500	1141	0	1085.45	95.1
		48	1046.00	91.7
		96	971.45	85.1

B. OBSERVATIONS

Clinical observations:

At the nominal concentration of 1136 and 2500 mg test item/L, the fish showed reduced activity and showed a tendency of staying at the bottom of the test aquarium after 24 h.
In comparison to the control group, no abnormal effects were seen at or below the concentration of 517 mg test item/L.

Table B.9.2.1-9: Lethal effects of glyphosate isopropylamine salt to rainbow trout

	Control					
Test item [mg/L]	**-**	**107**	**235**	**517**	**1136**	**2500**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**65.9**	**145**	**318**	**700**	**1540**
Glyphosate [mg a.e./L]	**-**	**48.8**	**107**	**236**	**519**	**1141**
Mortality (2-4 h) [%]	0	0	0	0	0	0
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	20
Mortality (72 h) [%]	0	0	0	0	0	40
Mortality (96 h) [%]	0	0	0	0	10	60

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

Assessment and conclusion

Assessment and conclusion by applicant:
In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for rainbow trout exposed to glyphosate isopropylamine salt was determined to be 2192 mg test item/L, corresponding to 1350 mg glyphosate isopropylamine salt/L (mg a.s./L) or 1001 mg glyphosate/L (mg a.e./L) (nominal). The NOEC was determined to be 517 mg test item/L, corresponding to glyphosate isopropylamine salt/L (mg a.s./L) or 236 mg glyphosate/L (mg a.e./L) (nominal).

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to glyphosate isopropylamine salt was determined to be >1001 mg a.e./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
The study was already considered in previous assessment (DAR/RAR).

The pH value at the highest tested concentration (pH 5.7 at 2500 mg glyphosate isopropylamine salt/L, equivalent to 1141 mg glyphosate acid) was slightly below the recommended range (recommended pH 6-8.5). The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options. Overall, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the pH should always be in a range required to maintain the health of the organisms tested (here 6.5-8.5). Given that pH is slighltly below the range the results are considered acceptable.
The new version of OECD 203 recommends a temperature in a range of 10-14°C (which is similar to the range given in OPPTS 850.1075). This range is exceeded (14.5 – 16.3°C) but still in accordance with the range given in the original TG203 of 13-17°C. This deviation is considered acceptable.
The recommended size (30-60 mm) is also slightly exceeded (mean 67 mm). This deviation is considered minor.

Considering the mean weight of the tested fish (up to 2.08 g/fish), loading was also exceeded at some concentrations. This does not seem to have affected the outcome of the study.
The study is valid according to validity criteria.
Analytic verifications are available for only three concentrations (out of 5) but appear consistent between them. The use of nominal is accepted by RMS.
Acute LC50 value for rainbow trout exposed to glyphosate isopropylamine salt =1001 mg a.e./L (nominal).
NOEC = 236 mg glyphosate acid/L (nominal)

Data point	CA 8.2.1/005
Report author	[redacted]
Report year	1990
Report title	Glyphosate technical: 96-hour Acute Toxicity Study (LC_{50}) in the Rainbow Trout
Report No	271631
Document No	-
Guidelines followed in study	OECD Guideline 203 (1983)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation according to the current guideline OECD 203:* *None*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid with restrictions (pH issues)

Executive Summary

The effects of glyphosate technical on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. Groups of ten fish each were exposed to glyphosate technical at concentrations of 95, 171, 309, 556, and 1000 mg a.s./L (nominal concentrations), corresponding to 87.7, 135, 188, 497 and 1019 mg a.s/L based on geometric mean measured concentrations. The number of surviving organisms and the occurrence of sub-lethal effects, as well as the measurement of dissolved oxygen, pH and water temperature were determined and recorded after 2, 24, 48, 72 and 96 hours after starting the exposure period. The concentrations of glyphosate in the test medium were determined at test initiation, and 2, 48 and 96 hours thereafter.

At test concentration of 87.7 mg a.s./L there was no fish mortality within the 96 hour duration of the study. Increasing the mean measured test concentration by a factor of about 1.5 to 135 mg a.s./L the mortality resulted in 100% within the first 48 h of exposure. All validity criteria according to the guideline OECD 203 were fulfilled.

The authors concluded that 96-h LC_{50} for *Oncorhynchus mykiss* exposed to glyphosate technical was estimated to be between 87.7 and 135 mg a.s./L based on (geometric) mean measured concentration. The NOEC after 96 h was 87.7 mg a.s./L. This study is considered valid.

Because of pH issues, the RMS considered that the 96-h LC_{50} for *Oncorhynchus mykiss* exposed to glyphosate technical was greater than 87.7 mg a.s./L

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	Solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9%

2. Vehicle of test material/media deionised water

3. Test organism:

Species:	Rainbow trout (*Salmo gairdneri*, currently known as *Oncorhynchus mykiss*)
Size of animal:	Weight 0.8 g (average) Length 42.4 mm (average)
Number of animals/dose level:	10 in each vessel
Mean loading rate (biomass per volume of test solution):	0.4-0.6 g/L
Supplier:	[redacted]

4. Environmental conditions:

Temperature:	11-12°C
pH:	2.8-8.1
Dissolved oxygen:	10.8-12.3 mg O_2/L (continuously aerated during the test)
Conductivity:	Not reported
Hardness:	250 mg $CaCO_3$
Illumination:	16 hours light/8 hours dark, 500-1500 lux
5. Experimental dates of work:	May 28th to June 1st 1990

B. STUDY DESIGN

Experimental treatments

The effects of glyphosate technical on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. Groups of ten fish each were exposed to glyphosate technical at nominal concentrations of 95, 171, 309, 556, and 1000 mg a.s./L. The test solutions were prepared by adding 1.425, 2.565, 4.635, 8.34, and 15 g test item to 15 L test medium (reconstituted water prepared according to the OECD Guideline) in the respective tanks. In addition fish were exposed to test medium without test substance (blank control).

Observations

The number of surviving organisms and the occurrence of sub-lethal effects, as well as the measurement of dissolved oxygen, pH and water temperature were determined and recorded after 2, 24, 48, 72 and 96 hours after starting the exposure period. The concentrations of glyphosate in the test medium were determined at test initiation, and 2, 48 and 96 hours thereafter.

Statistical calculations

The Logit-Model could not be used to estimate the LC_{50} value since the mortality rose from 0% to 100% within two test concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

At test initiation the concentrations of glyphosate in the test medium were in a range of 59.6 to 101.9% of nominal. At the end of the test, the concentration of glyphosate in the tank where all fish survived (95 mg a.s./L) was 104.6% of nominal. Therefore, the toxicity values are based on (geometric) mean measured concentrations. Analytical results are shown below.

Table B.9.2.1-10: Analytical results

Nominal concentration of glyphosate technical [mg a.s./L]	Time (hours)	Mean concentration of Samples A and B [mg a.s./L]	% of nominal	Geometric mean measured concentrations [mg a.s./L]
95	0	75.82	79.8	87.7
95	2	77.32	81.4	
95	48	95.33	100.3	
95	96	99.33	104.6	
171	0	124.4	72.7	135
171	2	108.5	63.5	
171	48	182.8	106.9	
309	0	184.1	59.6	188
309	2	192.6	62.3	
556	0	528.2	95.0	497
556	2	470.3	84.6	
1000	0	1019	101.9	1019
1000	2	1019.8	102.0	

At test concentration of 87.7 mg a.s./L there was no fish mortality within the 96 hour duration of the study. Increasing the mean measured test concentration by a factor of about 1.5 to 135 mg a.s./L the mortality resulted in 100% within the first 48 h of exposure. Based on these findings, the 96-h LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate technical was estimated to be between 87.7 and 135 mg a.s./L. The mortality in the control was 0%. The effects of glyphosate technical on mortality in rainbow trout are shown below.

Table B.9.2.1-11: Effects of glyphosate technical on mortality of rainbow trout

Nominal concentration of glyphosate technical [mg a.s./L]	**Control**	**95**	**171**	**309**	**556**	**1000**
Geometric mean measured concentrations of glyphosate technical [mg a.s./L]	**Control**	**87.7**	**135**	**188**	**497**	**1019**
Mortality (24 h) [%]	0	0	50	100	100	100
Mortality (48 h) [%]	0	0	100	100	100	100
Mortality (72 h) [%]	0	0	100	100	100	100
Mortality (96 h) [%]	0	0	100	100	100	100

B. OBSERVATIONS

At glyphosate technical concentrations of 309 and 1000 mg a.s./L, a sedimentation of the test material on the bottom of the tanks was observed.
Clinical signs were recorded at glyphosate technical concentrations of 171 and 309 mg a.s./L, whereas in the control and in the 95 mg a.s./L tanks no sub-lethal effects were recorded.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

Assessment and conclusion

Assessment and conclusion by applicant:

The 96-h LC_{50} for rainbow trout (*Oncorhynchus mykiss)* exposed to glyphosate technical was estimated to be between 87.7 and 135 mg a.s./L based on geometric mean measured concentrations. The NOEC after 96 h was 87.7 mg a.s./L.

Some precipitate observed at test concentrations 188 mg a.s./L and 1019 mg a.s./L. The validity criteria are fulfilled and so this study is considered valid and the acute LC_{50} value for rainbow trout exposed to glyphosate technical was estimated to be 87.7 - 135 mg a.e./L (geometric mean measured concentrations) and can be used in risk assessment.

Assessment and conclusion by RMS:

The pH values at the four highest nominal tested concentrations (171, 309, 556, 1000 mg glyphosate acid/L) were far below the recommended range (recommended pH 6-8.5). The actual pH values were of 2.8 at 1000 mg/L and 4.0 at 171 mg/L (with intermediate pH values at intermediate concentrations). This deviation was negatively correlated with increasing concentrations of glyphosate indicating that this pH decrease was due to the test item (intrinsic biochemical characteristic of glyphosate acid). It should be noted that the study was already considered in previous assessment (DAR/RAR).

The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to

distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options.
In Schweizer, M. et al (2019) (CA 8.2.2.1/006 see addendum of B.9 (AS) on general literature review), zebrafish (*Danio rerio*) embryos acutely exposed to glyphosate at concentrations between 1.69 and 1690.7 mg glyphosate/L for 96 hours post fertilization in buffered and unbuffered situation. A particular attention was paid on pH from 3 to 4. The study demonstrates that the severe effects detected seemed to be mainly caused by a low (glyphosate induced) pH.
Overall, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the pH should always be in a range required to maintain the health of the organisms tested (here 6.5-8.5). At 95 mg/L (nominal, corresponded to 87.7 mg a.e./L) , the pH (5.6) was slightly below the recommended range and no mortality was observed. RMS therefore proposed to set the endpoint at this value.

This study is considered valid as the validity criteria are met but with restrictions due to pH issue.
96-h LC50 for rainbow trout (*Oncorhynchus mykiss*) > 87.7 mg glyphosate acid/L (geometric mean measured concentrations).
NOEC = 87.7 mg glyphosate acid/L (geometric mean measured concentrations).

Data point:	CA 8.2.1/006
Report author	[redacted]
Report year	1981
Report title	Acute Toxicity of MON 0139 (lot LURT 12011) ([redacted]-81-072) to Rainbow Trout (*Salmo gairdneri*)
Report No	27202
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *Major:* *- No analytical verification of test concentrations* *none* *Minor:* *- Fish were acclimatised 48 hours prior to the test (7 days are required)* *- Fish lengths 25 - 31 mm (30 to 60 mm is required)* *- pH of the highest concentration (4.6) was not with the specified range of 6.0 - 8.5.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Executive Summary
The effects of glyphosate isopropylamine salt (MON 0139) on the rainbow trout (*Salmo gairdneri*, currently known as *Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L, corresponding to 62.5, 112, 200, 350 and 625 mg glyphosate isopropylamine salt/L (mg a.s./L) or 46.3, 83.3, 148, 259 and 463 mg glyphosate/L (mg a.e./L). In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. No mortality was observed at any of the test concentrations up to and including 1000 mg test item/L, corresponding to 625 mg a.s./L or 463 a.e./L (nominal).

In a static acute fish toxicity test, the LC_{50} (96 h) for rainbow trout (*Salmo gairdneri*) exposed to glyphosate isopropylamine salt (MON 0139) was determined to be >1000 mg test item/L (nominal), corresponding to >625 mg a.s./L or 463 mg a.e./L (nominal).
The validity of the present study according to OECD guideline 203 is questionable, since the analytical part of the study was not performed and/or reported. The study is considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt (MON 0139)
Description:	Light yellow liquid
Lot/Batch #:	LURT 12011
Purity:	62.49%

2. Vehicle of test material/media and positive control: Vehicle: Deionised water
Positive control: Antimycin A

3. Test organism:

Species:	Rainbow Trout (*Salmo gairdneri)*
Age:	At least 14 days old
Size:	Length: 27 mm (mean)
Body weight:	0.22 g (mean)
Loading:	10 test individuals for 15 L test solution (=0.146 g/L)
Source:	[redacted]
Diet/Food:	Daily with Standard commercial fish food (Rangen's) except 48 prior to the test
Acclimation period:	48 hours prior to the test initiation

4. Environmental conditions:

Temperature:	12 ± 1°C
Photoperiod:	16 h light
pH:	7.0
Dissolved oxygen:	9.8 mg/L
Conductivity:	Not stated
Hardness:	45 mg $CaCO_3$/L.

5. Experimental dates of work: March 10th to March 14th 1981

B. STUDY DESIGN

Experimental treatments: Based on the results of a 48-h range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L. In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. There was one vessel per treatment, containing ten fish in 5 gallon (appr. 19 L) glass vessels containing 15 L test medium.

Observations: The fish mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after the test initiation. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. The weight and length of the test fish were measured.

Statistical calculations: LC_{50} values were calculated using computer program by Stephan et al. (1978). (Stephan, C.E., K.A. Busch, R. Smith, J. Burke and R.W,. Andrew. 1978. A computer program for calculating an LC50. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978)

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification of the tested concentrations was conducted or reported.

The LC_{50} values are given below based on nominal concentrations.

Table B.9.2.1-12: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50}	>1000	>625	>463

B. OBSERVATIONS

There was no mortality observed at any of the test concentrations up to and including 1000 mg test item/L. For the reference product Antimycin A, the LC_{50} was determined to be 0.000030 mg/L. The dissolved oxygen concentration which stayed between 40 and 100 % saturation was considered adequate for testing. The pH values dropped with increasing test concentrations.

Table B.9.2.1-13: Lethal effects of glyphosate isopropylamine salt (MON 0139) to *Salmo gairdneri*

	Control					
Test item [mg/L]	**-**	**100**	**180**	**320**	**560**	**1000**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**62.5**	**112**	**200**	**350**	**625**
Glyphosate [mg a.e./L]	**-**	**46.3**	**83.3**	**148**	**259**	**463**
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	0
Mortality (72 h) [%]	0	0	0	0	0	0
Mortality (96 h) [%]	0	0	0	0	0	0

The following validity criteria according to the OECD 203 (2019) were fulfilled:

- The dissolved oxygen concentration was maintained ≥60 % of the air saturation value (ranging from 9.9 to 9.4 mg/L through the study).
- The control mortality was lower than 10 % at the end of the study.

The following validity criterion according to the OECD 203 (2019) was not fulfilled:

- No analytical measurement of the test concentrations was reported.

The following points deviated from current guideline too:

- Fish were acclimatised 48 hours prior to the test instead of the 7 day requirement.
- Fish length varied between 25-31 mm instead of 30 to 60 mm required.
- Observations occurred after 24 h, 48 h and 96 h. The requirements are the following: a minimum of 2 observations within the first 24 hours of the study and on days 2-4 of the test, all vessels with living fish inspected twice per day (preferably early morning and late afternoon to best cover the 24-hour periods).
- The pH in the highest concentration (pH 4.6) was outside of the accepted range of 6.0-8.5 so the stock solution should have been adjusted to lie within this specified range. No pH measurements are available at 180, 320 and 560 mg/L.

These deviations may affect the outcome of the study, so the validity of the study is questionable.

Assessment and conclusion

Assessment and conclusion by applicant:

In a static acute fish toxicity test, the LC_{50} (96 h) for rainbow trout (*Salmo gairdneri*) exposed to glyphosate isopropylamine salt (MON 0139) was determined to be >1000 mg test item/L (nominal), corresponding to >625 mg a.s./L or >463 mg a.e./L (nominal).

Not all validity criteria according to the OECD 203 (2019) were fulfilled since the analytical part of the study was not performed and/or reported. Taking also into account the minor deviations, that may affect the outcome of the study, the study is therefore considered as supportive.

Assessment and conclusion by RMS:

As summarised above, major deviations from the current OECD 203 guideline (2019) were highlighted in this study.

LC50 (96 h) for rainbow trout (*Salmo gairdneri*) exposed to glyphosate isopropylamine salt (MON 0139) >1000 mg test item/L (nominal), corresponding to >625 mg a.s./L or >463 mg a.e./L (nominal). The NOEC is set to 463 mg glyphosate acid/L (nominal).

No analytical verification of test concentrations are available. For this reason, this study should be considered unreliable. However, as IPA concentrations were maintained in similar studies, RMS considers the results informative even if not robust enough to derive a reliable endpoint to be used for risk assessment.

This study is informative only.

Data point:	CA 8.2.1/007
Report author	[redacted]
Report year	1978
Report title	Acute Toxicity of Technical Glyphosate ([redacted]-78-165) to Rainbow Trout (*Salmo gairdneri*)
Report No	[redacted] 78-165
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *Major:* *- No analytical verification of test concentrations* *Minor:* -
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Supportive

Executive Summary

The acute effects of glyphosate technical on rainbow trout (*Salmo gairdneri* – currently known as *Oncorhynchus mykiss)* were evaluated in a 96-hour static toxicity test. A definitive toxicity test was performed using nominal concentrations of 42, 87, 120, 180, 240 and 420 mg test item/L, corresponding to 34.9 72.2, 99.6, 149, 199 and 349 mg glyphosate technical/L(mg a.s./L), following a range-finding test. A control group was exposed to deionised water and a reference treatment group exposed to Antimycin A were also tested.

The mortality of fish was recorded at 24, 48 and 96 hours after test initiation. At 24 hours, there was 100% mortality in the 240 and 420 mg test item/L treatment groups. At 48 hours, there was 100% mortality in the 180 mg test item/L treatment group. At 96 hours, in the 120 mg test item/L group there was 100% mortality recorded, 40% mortality at 87 mg test item/L and no mortality in the control group or the lowest concentration (42 mg test item/L). The LC_{50} (96 h) was determined to be 86 mg test item/L, corresponding to 71.4 mg a.s./L (nominal). The NOEC was determined to be 42 mg test item/L, corresponding to 34.9 mg a.s./L (nominal).

According to the current OECD 203 test guideline, despite the control validity criteria of <10% mortality being achieved, there was no chemical analysis performed to confirm glyphosate concentration in the test media. The test would therefore not be considered valid against the current criteria. Within the context of the Annex I renewal of glyphosate, this study may only be considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHI-162
Purity:	83.0%

2. Vehicle of test material/media and positive control: Vehicle: deionised water
Positive control: Antimycin A

3. Test organism:

Species:	Rainbow trout (*Salmo gairdenri*)
Age:	Not stated
Size:	Length: 39 mm (mean, reference toxicant group: 34 mm)
Loading:	10 individual fish per vessel (19 L glass vessel) in 15 L test solution
Source:	[redacted]
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	0.58 g (mean, reference toxicant group = 0.55g)

4. Environmental conditions:

Temperature:	12 ± 1°C
Photoperiod:	Not stated
pH:	7.0 – 7.2 (control); 4.4 – 5.8 (120 mg test item/L)
Dissolved oxygen:	7.6 – 8.7 mg/L
Conductivity:	Not stated
Hardness:	45 mg $CaCO_3$/L

5. Experimental dates of work: July 29th to August 2nd 1978

B. DESIGN AND METHODS

Experimental treatments: Following a range-finding test, a definitive test was conducted at nominal test concentrations of 42, 87, 120, 180, 240 and 420 mg test item/L, corresponding to 34.9, 72.2, 99.6, 149, 199 and 398 mg glyphosate a.s./L, in a static test setup. The test item was dissolved directly into dilution water. A control group was also prepared using fish exposed to dilution water only (soft reconstituted water using deionised water).

A reference toxicant test was conducted in parallel with fish exposed to Antimycin A at rates between 0.000024 – 0.00032 mg /L. Acetone was used to prepare the reference toxicant media.

A single replicate vessel was prepared per treatment, control and reference toxicant group.

Observations: Mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after test initiation in the glyphosate exposure test and additionally at 72 hours in the reference toxicant test. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. Weight and length of the test fish were equally measured.

Statistical calculations: LC_{50} values were calculated along with the 95% confidence limits using Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

At and above nominal concentrations of 120 mg test item/L, no fish survived. At a nominal concentration of 87 mg test item/L, 40% mortality was recorded whereas no mortality was observed at the lowest test concentration of 42 mg test item/L. For the highest concentration of the reference product Antimycin A (0.00032 mg/L), 100% mortality was observed 24 hours after the test initiation.

Table B.9.2.1-14: Lethal effects of glyphosate to rainbow trout

Test item [mg/L]	**C**	**42**	**87**	**120**	**180**	**240**	**480**
Glyphosate technical [mg a.s./L]	**-**	**34.9**	**72.2**	**99.6**	**149**	**199**	**398**
Mortality (24 h) [%]	0	0	0	0	70	100	100
Mortality (48 h) [%]	0	0	0	60	100	100	100
Mortality (96 h) [%]	0	0	40	100	100	100	100

C = Control

The LC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.1-15: Endpoints

Endpoints (96 h)	**Test item [mg/L]**	**Corresponding glyphosate technical concentration [mg a.s./L]**	**Reference [mg/L]**
LC_{50} (95% C.I.)	86 (70 - 106)	71.4 (58.1 - 88.0)	4.2×10^{-5} (3.6×10^{-5} - 4.9×10^{-5})
NOEC	42	34.9	-

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a static acute fish toxicity study of glyphosate, the LC_{50} (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to the glyphosate technical was determined to be 86 mg test item/L, corresponding to 71.4 mg a.s./L (nominal). The NOEC was determined to be 42 mg test item/L, corresponding to 34.9 mg a.s./L (nominal).

No chemical analysis was performed to confirm glyphosate concentration in the test media. The test would therefore be considered supportive for risk assessment purposes.

Assessment and conclusion by RMS:

As summarised above, major deviations from the current OECD 203 guideline (2019) were highlighted in this study. The study was already considered in previous assessment (DAR/RAR).

The pH was below the recommended range at 87 and 120 mg/L (pH 5.6 and 4.4 respectively) and was not measured at higher concentrations.

This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH

6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options. The mortality observed at these concentrations (and higher) can be explained by the acidity of the test solution. RMS considered that results up to the concentration of 87 mg/L (nominal) can be considered to be not pH induced. The proposed LC50 is seen to be conservative as include pH effects at highest doses.

No analytical verification of test concentrations are available. For this reason, this study should be considered unreliable. However, as concentrations were maintained in similar studies, RMS considers the results informative even if not robust enough to derive a reliable endpoint to be used for risk assessment.

LC50 (96 h) for rainbow trout (Oncorhynchus mykiss) exposed to the glyphosate technical = 86 mg test item/L, corresponding to 71.4 mg glyphosate acid (nominal). The NOEC is set to 34.9 mg glyphosate acid/L (nominal).
This study is only informative.

Data point:	CA 8.2.1/008
Report author	[redacted]
Report year	1972
Report title	Four-day static fish toxicity studies with CP 67573 in rainbow trout and bluegills.
Report No	[redacted]-72-104
Document No	-
Guidelines followed in study	Not mentioned.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *Major:* *- No analytical verification of test concentrations* *- 60% of the air saturation was not maintained throughout the test.* *Minor:* *- Oxygen, pH and temperatures were not daily measured.* *- The weight of the fish were not provided, so the loading cannot be calculated.* *- The length of bluegill ranged between 3.5 and 7.5 cm.* *- Temperature of bluegill test was 18°C.*
Previous evaluation	Rainbow trout: Yes, accepted in RAR (2015) Bluegill: Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Not reliable

Executive Summary
The acute effects of glyphosate acid (CP 67573) to rainbow trout (*Oncorhynchus mykiss*) and bluegills (*Lepomis macrochirus*) were evaluated in a 96-hour static toxicity tests. These tests were conducted at nominal test concentrations of 10, 18, 32, 56 and 78 mg a.s./L for rainbow trout and 32, 56, 56, 70, 85

and 100 mg a.s./L for bluegill. A control and a toxic reference item (Toxaphene) were also included in the test. Ten fish were exposed in the control and in each treatment. All fish were observed at daily intervals over the 96-hour study duration, with mortality and sub-lethal signs of toxicity recorded. Dissolved oxygen and pH values were measured for all solutions in which mortalities occurred. The temperature was maintained at 13°C for rainbow trout and 18°C for bluegills. Glyphosate acid (CP 67573) was found to have a very low solubility in water. No analytical measurements were performed. Only one of the three validity criteria according to the guideline OECD 203 was fulfilled (control mortality < 10%).
The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid (CP 67573) was determined to be 38 mg a.s./L with a 95% confidence interval of 25 to 56 mg a.s./L.
The 96-hour LC_{50} value for the bluegills exposed to glyphosate acid (CP 67573) was determined to be approximately 78 mg a.s./L (95% confidence interval was not recorded).

The study was previously considered valid (RAR 2015) and was part of the list of endpoints, being the lowest available fish acute toxicity endpoint. However, as no analytical verification of test item was performed and oxygen levels decreased below 60%, this are major deviations to the guideline. Taking also into account that some minor deviations were pointed out, the study is not considered valid according to OECD 203.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid (CP 67573)
Description:	Low solubility in water
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle of test material/media and positive control:

Vehicle: reconstituted water
Positive control: Toxaphene

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
	Bluegill (*Lepomis macrochirus*)
Age:	Juvenile
Size:	Length: 35-75 mm
Body weight of the animals:	Not reported
Loading:	Not reported
Source:	Not reported
Diet/Food:	Brine shrimp or purina trout chow no feeding for 3 days prior to test
Observation period:	14 days prior to experimental use
Acclimation period:	24 hours

4. Environmental conditions:

Temperature:	13°C for rainbow trout and 18°C for bluegill
Photoperiod:	Not reported
pH range from start to 96h	

for rainbow trout:	Control: 7.0 - 7.3 18 mg/L: 6.2 32 mg/L: 6.0 – 6.4 56 mg/L: 5.5 – 6.2 78 mg/L: 4.0
for bluegill:	Control: 6.9 – 7.1 85 mg/L: 4.0 100 mg/L: 3.9 – 4.1
Dissolved oxygen range from start to 96h	
for rainbow trout:	Control: 5 - 7.6 mg O_2/L 18 mg/L: 0.8 mg O_2/L 32 mg/L: 3.0 – 3.4 mg O_2/L 56 mg/L: 2.6 – 6.0 mg O_2/L 78 mg/L: 6.7 mg O_2/L
for bluegill:	Control: 4.1 – 6.8 mg O_2/L 85 mg/L: 6.8 mg O_2/L 100 mg/L: 7.1 – 8.2 mg O_2/L
Conductivity:	Not recorded
Hardness:	Not recorded
5. Dates of experimental work:	Not reported

B. STUDY DESIGN

Experimental treatments

The toxicity test was performed with glyphosate acid (CP 67573) at nominal concentrations of 10, 18, 32, 56 and 78 mg a.s./L for rainbow trout and 32, 56, 70, 85 and 100 mg a.s./L for bluegill, prepared using reconstituted water. The bioassay vessels prepared for the control and at each treatment level, were lined with disposable polyethylene bags and then filled with 12.5 L of reconstituted water, with ten fish then added to each vessel. After an acclimation period of 24 hours, the test material was added directly to the vessels containing the fish. The tests were conducted under static test conditions. A negative control (water only) was also prepared. Toxaphene was used as toxic reference item and dispensed in the form of a 0.01% w/v solution in acetone.

Observations

Fish in all vessels were observed for 96 hours after the introduction of the test material directly to the vessels, with sublethal effects (e.g. quiescence, mucosa shedding) and mortality recorded daily. The pH-value and oxygen saturation of test solutions were measured in all solutions in which mortalities occurred. Hardness and conductivity of the test water were not measured. Analytical measurements were not performed.

Statistical calculations

The four-day median tolerance level TL_{50} (equivalent to an LC_{50} value) and corresponding 95% confidence intervals, were calculated using the technique of Litchfield, J. T., Jr. and Wilcoxon, F., "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharm. & Exp. Ther. 96, 99 (1949).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: No analytical verification of test concentrations was performed.

The 96 hour LC_{50} values are presented below.

Table B.9.2.1-16: Endpoints

Endpoints (96h)	Glyphosate acid (CP 67573) [mg a.s./L]
Rainbow trout LC_{50} (95% CI)	38 (25 – 56)
Bluegill LC_{50} (95% CI)	≈ 78 (n.d.)

CI= Confidence interval
n.d.= not determined

The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid (CP 67573) was determined to be 38 mg a.s./L with a 95% confidence interval of 25 to 56 mg a.s./L.

The 96-hour LC_{50} value for bluegill exposed to glyphosate acid (CP 67573) was determined to be approximately 78 mg a.s./L (95% confidence interval was not recorded).

B. OBSERVATIONS

For the rainbow trout:
At test concentrations of 18 and 32 mg a.s./L three fish died within 96 hours of exposure. At test concentration of 56 mg a.s./L four fish died within 96 hours of exposure. Increasing the test concentration by a factor of about 1.4 (78 mg a.s./L) the mortality resulted to be 100% within the first 24 hours of exposure.
Sublethal effects of quiescence, swimming against tank side on bottom, patchy shedding of external mucosa were observed within the 6 hours after exposure at 78 mg test item/L and within 24 hours at concentrations up to 18 mg test item/L. There were no recovery until the end of the test when sublethal effects were detected.
The fish were in the recommended range length of 3 to 6 cm (actual values ranged between: 3.5 and 7.5 cm). The water quality parameters were not recorded except for control pH which was within the OECD 203 specifications of 6 to 8.5 (actual value: 7.6). The levels of pH declined with increasing concentration of the test item, with a pH of 4.0 being recorded at the highest rate. The biological observations recorded during the test are presented below.

Table B.9.2.1-17: Effects of CP 67573 to rainbow trout

Nominal concentration of glyphosate acid [mg a.s./L]	Number of survivor/observed symptoms [1]					96 h Survival %
	1-6 h	24 h	48 h	72 h	96 h	
Control	10/no	10/no	10/no	10/no	10/no	100
10	10/no	10/no	10/no	10/no	10/no	100
18	10/no	10/Q	10/Q	10/Q	7/Q	70
32	10/no	10/Q	10/Q	10/Q	7/Q	70
56	10/no	9/Q	8/Q, S	7/Q, S	6/Q, S	60
78	10/Q, S, E, P	0	0	0	0	0

[1] Q = quiescence, S = swimming against tank side on bottom, E = external mucosa shedding and P = patchy

For the bluegill:
No mortality occurred up the concentration of 70 mg a.s./L within the 96 hours of exposure. At test concentration of 85 mg a.s./L four fish died within 96 hours of exposure. At the highest test concentration of 100 mg a.s./L, the mortality resulted in 100% within 72 hours of exposure.
Sublethal effects of quiescence, light discoloration, external mucosa shedding or patchy behaviour were observed at the concentration of 56 mg a.s./L and higher. There were no recovery until the end of the test when sublethal effects were detected.
The fish were not in the recommended range length of 1 to 3 cm (actual values ranged between: 3.5 and 7.5 cm). The water quality parameters were not recorded except for control pH which was within the OECD 203 specifications of 6 to 8.5 (actual value: 6.8). The levels of pH declined with increasing concentration of the test item. The temperature was not in the required range of 21 to 25°C (actual value: 18°C). The biological observations recorded during the test are presented below.

Table B.9.2.1-18: Effects of CP 67573 to bluegill

Nominal concentration of glyphosate acid [mg a.s./L]	Number of survivor/observed symptoms [1]					96 h Survival %
	1-6 h	24 h	48 h	72 h	96 h	
Control	10/no	10/no	10/no	10/no	10/no	100
32	10/no	10/no	10/no	10/no	10/no	100
56	10/no	10/Q	10/Q	10/Q	10/Q	100
70	10/no	10/Q, L	10/Q, L	10/Q, L	10/Q, L	100
85	10/Q, L, E, P	10/ Q, L, E, P	6/ Q, L, E, P	6/ Q, L, E, P	6/ Q, L, E, P	60
100	10/Q, L, E, P	7/ Q, L, E, P	4/ Q, L, E, P	0	0	0

[1] Q = quiescence, L = light discoloration, E = external mucosa shedding and P = patchy

General observations:
The test material, CP 67573, was found to have a very low solubility in water. At higher dose levels (56 mg a.s./L and upward) the test material displayed a very high acidity. Primarily those fish which came into direct contact with the test material (as it dropped to the bottom) were more affected.

The following points deviated from the current guideline:
- Oxygen, pH and temperatures were not daily measured.
- The weight of the fish were not provided, so the loading cannot be calculated.
- The length of bluegill ranged between 3.5 and 7.5 cm.
- Temperature of bluegill test was 18°C.

Validity criteria

In order to consider the test to be valid according to OECD 203, the following conditions should be fulfilled:

- Control mortality should not exceed 10% at the end of the exposure. No mortality was recorded in the control for both tests.
- The dissolved oxygen concentration should be ≥60 % of the air saturation value in all test vessels throughout the exposure. Air saturation was not reported. The dissolved oxygen values varied from 7.6 to 0.8 mg O_2/L, for rainbow trout. The dissolved oxygen values varied from 8.2 to 4.0 mg O_2/L, for bluegill. Hence, the dissolved oxygen concentration was not steady throughout the test.
- Analytical measurement of test concentrations is compulsory, however no analytical measurement was performed.

According to the current validity criteria of OECD 203 guideline, this study is not valid. The dissolved oxygen concentration above 60% of air saturation and evidence that the concentration of the chemical being tested has been satisfactorily maintained (at least 80% of the nominal concentration) throughout the test, cannot be concluded.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 96-hour LC_{50} value for rainbow trout exposed to glyphosate acid (CP 67573) was determined to be 38 mg a.s./L with a 95% confidence interval of 25 to 56 mg a.s./L.
The 96-hour LC_{50} value for bluegill exposed to glyphosate acid (CP 67573) was determined to be approximately 78 mg a.s./L (95% confidence interval was not recorded).

The study was previously considered valid (RAR 2015) and was part of the list of endpoints, being the lowest available fish acute toxicity endpoint. However, as no analytical verification of test item was performed and oxygen levels decreased below 60%, this are major deviations to the guideline. Taking also into account that some minor deviations were pointed out, the study is not considered valid according to OECD 203. Other valid studies with comparable results are available. This study is not considered acceptable for risk assessment.

Assessment and conclusion by RMS:
Severe drawbacks were highlighted in this study (for both tested species).
For these reasons, this study should be considered unreliable.
Despite the absence of analytical verification, a dose response is observed (for trout). Nevertheless RMS considers the results not robust enough to derive a reliable endpoint to be used for risk assessment due to the drawbacks that were identified.
RMS notes that the levels of pH declined with increasing concentration of the test item, with a pH of 4.0 being recorded at the highest rate 78 mg/L. This deviation was negatively correlated with increasing concentrations indicating that this pH decrease was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). To be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options. It should be noted that the study was already considered in previous assessment (DAR/RAR). The mortality observed at the highest concentration (78 mg/L) can be explained by the acidity of the test solution.

The pH values were of 5.5-6.0 at 56 mg/L. This is still below the recommended range (6-8.5) however this slight deviation does not explain the 40% mortality that were observed at this rate.

The results available are considered not reliable for both tested species (trout and bluegill).

Data point:	CA 8.2.1/009
Report author	[redacted]
Report year	1995
Report title	Glyphosate acid: Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*)
Report No	[redacted]5553/B
Document No	-

Guidelines followed in study	US EPA Guideline, FIFRA subdivision E, section 71-1.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *None*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid with restrictions (pH issues)

Executive Summary

The acute effects of glyphosate acid to bluegill sunfish (*Lepomis macrochirus*) was evaluated in a 96-hour static toxicity test performed at nominal test concentrations of 10, 18, 32, 56, 100 and 180 mg a.s./L. A dilution water only control was also included in the test. Ten fish were exposed in the control and in each treatment. All fish were observed at daily intervals over the 96 hour study duration, with mortality and sub-lethal signs of toxicity recorded.

Dissolved oxygen, pH and temperature were measured daily in each test vessel. Samples of control and test media were analysed for glyphosate acid at 0 hours (before fish addition) and after 48 and 96 hours. Glyphosate acid was not detected in the control group. The overall mean measured concentrations of glyphosate acid in the treatment groups ranged from 94.4 to 97% of nominal concentrations.

There was no fish mortality or sublethal effects observed in the control group, and in the 10, 18 and 32 mg a.s./L treatments. By 96 hours, there was 90% mortality in the 56 mg a.s./L treatment and 100% mortality in the 100 and 180 mg a.s./L treatments. All validity criteria according to the OECD guideline 203 were fulfilled.

The authors concluded that the 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg a.s./L (nominal concentration) with 95% confidence interval of 35 to 66 mg a.s./L. The NOEC after 96 hours was 32 mg glyphosate acid/L (nominal concentration). The study is considered valid.

Due to pH issues, RMS considered that the LC50 should be greater than 32 mg glyphosate acid/L. The NOEC is set at 32 mg glyphosate acid/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Glyphosate acid
Description: White solid
Lot/Batch #: P24
Purity: 95.6% a.s.

2. Vehicle of test material/media: dechlorinated, filtered tap water

3. Test organism:

Species: Bluegill sunfish (*Lepomis macrochirus*)

Age:	Juvenile
Size:	Length: 26 to 35 mm (mean = 30 mm)
Body weight:	0.29 to 0.96 g (mean = 0.54 g)
Loading:	10 test individuals for 20 L test solution
Source:	[redacted]
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimation period:	19 days at 22°C prior to the test initiation

4. Environmental conditions:

Temperature:	22 ± 1°C
Photoperiod:	16 hours with 20 min transition period
pH:	Control (start – 96 h): 7.3–6.8 10 mg/L (start – 96 h): 5.9 – 6.4 18 mg/L (start – 96 h): 5.2 – 5.8 32 mg/L (start – 96 h): 4.6 – 4.8 56 mg/L(start – 96 h): 3.8 – 3.9 100 mg/L (start – 24 h): 3.4 180 mg/L (start – 24 h): 3.1
Dissolved oxygen:	6.2 – 9.0 mg/L
Conductivity:	100 µS/cm
Hardness:	16.0 mg $CaCO_3$/L.

5. Dates of experimental work: November 20th to November 24th 1995

B. STUDY DESIGN

Experimental treatments: The acute toxicity test was performed at nominal concentrations of 10, 18, 32, 56, 100 and 180 mg a.s./L prepared using filtered and dechlorinated tap water treated with ultra violet steriliser. The test was conducted under static test conditions (no media renewal). A negative control group (dilution water only) was also prepared. A single vessel was prepared for the control and each test media group, each containing ten fish (27.5 L borosilicate glass vessels containing 20 L test medium).

Observations: Fish in all vessels were observed for sublethal effects and mortality after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. Samples of test media were analysed for glyphosate acid content using HPLC analysis at test initiation and after 48 and 96 hours.

Analytical procedures: Samples were taken from the centre of the test solutions. Glyphosate acid concentrations in the test solutions were determined at 0, 48 and 96 hours by high performance liquid chromatography method using a fluorescence detector. The samples were quantified against standards of glyphosate acid. Prior to analysis, samples and standards were derivatised using flourenylmethyl chlorformate, to prepare a fluorescing derivate.

Statistical calculations: The 96 hour LC_{50} values and 95% confidence intervals were calculated using non-linear interpolation. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The measured concentrations of glyphosate acid in fresh media at test initiation ranged between 96.9 and 110 % of nominal. In aged test media at 96 hours, mean measured glyphosate acid concentrations ranged between 94.4 and 97.0 % of nominal. At 100 and 180 mg a.s./L, no chemical analysis was performed at 96 hours, as all there was 100 % fish mortality within the first 24 hours following addition.

Table B.9.2.1-19: Analytical results

Nominal concentration of glyphosate acid [mg a.s./L]	Measured concentration of glyphosate acid [mg a.s./L] at 0 hours	Measured concentration of glyphosate acid [mg a.s./L] at 48 hours	Measured concentration of glyphosate acid [mg a.s./L] at 96 hours	% of nominal
Dilution water control	< 0.023	< 0.023	< 0.023	-
10	11	10	9.7	100
18	18*	19*	17*	100
32	31	33	31	100
56	55	57	54	98
100	99	100	-	100
180	180	180	-	100

- Not sampled, 100% mortality on previous sampling occasion
* mean of triplicate analysis

As measured concentrations of glyphosate acid were between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item. The limit of detection was 0.023 mg/L.

The 96 h LC_{50} value and corresponding NOEC value based on nominal concentrations are given below.

Table B.9.2.1-20: Endpoints

Endpoints (96h)	Glyphosate acid [mg a.s./L]
LC_{50} (95% CI)	47 (35- 66)
NOEC	32

CI= Confidence interval

B. OBSERVATIONS

There were no mortalities in the control or the 10, 18 and 32 mg a.s./L treatments. At 56 mg a.s./L, there was 90% mortality. There was 100% mortality at 100 mg a.s./L and higher test concentrations that occurred after 24 hours.

There was a strong negative correlation between pH value and test item concentrations observed. At 56 mg a.s./L, the pH was reduced to 3.8 and lower.

The biological observations recorded during the test are presented in the table below.

Table B.9.2.1-21: Effects of glyphosate acid to *Lepomis macrochirus*

Nominal concentration of glyphosate acid [mg a.s./L]	Number of dead fish / number of fish with intoxication symptoms [1] and observed symptoms			
	24 h	48 h	72 h	96 h
Control	0 / 0	0 / 0	0 / 0	0 / 0
10	0 / 0	0 / 0	0 / 0	0 / 0
18	0 / 0	0 / 0	0 / 0	0 / 0
32	0 / 0	0 / 0	0 / 0	0 / 0
56	4 / 4	8 / 8	9 / 9	9 / 9
100	* / *	* / *	* / *	* / *
180	* / *	* / *	* / *	* / *

[1] Dead fish are added to the sum of fish with symptoms
* / *All fish dead

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusion

Assessment and conclusion by applicant:
The 96 hour LC_{50} value for bluegill sunfish (*Lepomis macrochirus)* exposed to glyphosate acid was 47 mg a.s./L (nominal) with a 95% confidence interval of 35 to 66 mg a.s./L. The 96 hour NOEC was 32 mg a.s./L (nominal).

This study is considered valid and the acute LC_{50} value for bluegill sunfish exposed to glyphosate acid was 47 mg a.s./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
The pH values at the three highest tested concentrations (56, 100,180 mg glyphosate acid/L) were far below the recommended range (recommended pH 6-8.5). The actual pH values were of 3.13 at 180 mg/L and 3.82 at 56 mg/L (with intermediate pH values at intermediate concentration of 100 mg/L). This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of glyphosate acid). It should be noted that the study was already considered in previous assessment (DAR/RAR).

The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options.
In Schweizer, M. et al (2019) (CA 8.2.2.1/006 see addendum of B.9 (AS) on general literature review), zebrafish (*Danio rerio*) embryos acutely exposed to glyphosate at concentrations between 1.69 and 1690.7 mg glyphosate/L for 96 hours post fertilization in buffered and unbuffered situation.

A particular attention was paid on pH from 3 to 4. The study demonstrates that the severe effects detected seemed to be mainly caused by a low (glyphosate induced) pH.
Overall, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the pH should always be in a range required to maintain the health of the organisms tested (here 6.5-8.5). Therefore, as pH are below is 4 for test concentration equal or greater than 180mg/L, it can not be excluded that the high mortality observed at these concentrations was due to the acidity of the test solutions caused by high concentrations of glyphosate acid. At 10, 18 and 32 mg/L, pH was of 5.90, 5.18 and 4.52 respectively. As no mortality occurred, it is proposed to set the endpoint at the highest tested dose that did not induce mortality.

This study is considered valid with restriction (pH issues).

Acute LC50 value for bluegill sunfish exposed to glyphosate acid > 32 mg glyphosate acid/L (nominal). The NOEC is 32 mg glyphosate acid/L (nominal)

Data point	CA 8.2.1/010
Report author	[redacted]
Report year	1991
Report title	Glyphosate technical: 96-Hour Acute Toxicity Study (LC_{50}) in the Bluegill Sunfish
Report No	271642
Document No	-
Guidelines followed in study	EEC directive 92/69, Part C.1 OECD guidelines No. 203 (1992) EPA 540/9-82-024
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviation according to the current guideline OECD 203: -none.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
The effects of glyphosate technical on bluegill sunfish (*Lepomis macrochirus*) were evaluated in a 96-hour static toxicity test conducted with nominal test concentrations of 59.3, 88.9, 133.3, 200 and 300 mg a.s./L, corresponding to 43.3, 91.0, 119, 173 and 243 mg a.s./L based on geometric mean measured concentrations. Furthermore, a blank control and a stability control with 300 mg a.s./L (nominal) was tested. Ten fish were exposed to each treatment.

Mortality and sublethal effects were recorded 2, 24, 48, 72 and 96 hours after the start of the test. Prior to the start of the test, all animals were weighed and measured. Dissolved oxygen, pH and temperature were also measured and recorded prior to addition of the test article and 2, 24, 48, 72 and 96 hours after

the start of the test in each test chamber. Concentration of the test item was determined by HPLC in the untreated control and for all test concentrations shortly after addition of the test item and 2, 48 and 96 hours after the start of the test except from test concentrations with 100% mortality. During the test period of 96 hours the fish were exposed to mean concentrations ranging between 59.6 and 144.2 % (average for test concentrations of 59.3 to 300 mg test item/L) of nominal concentration.

No mortality or sublethal effects occurred at geometric mean measured concentrations of up to 119 mg/L. The mortality was 100% at the 173 mg a.s./L test concentration, based on geometric mean measured concentration. At these high test concentrations the pH was very low (3.2 – 3.6). All validity criteria according to the guideline OECD 203 were fulfilled.

The authors proposed a LC_{50} (96 h) for rainbow trout exposed to glyphosate technical ranged between 133.3 mg a.s./L and 200 mg a.s./L (nominal), corresponding to 119 mg a.s./L and 173 mg a.s./L (geometric mean measured). The 96 hour NOEC was 133.3 mg a.s./L (nominal), corresponding to 119 mg a.s./L (geometric mean measured).

RMS concluded that the study is supportive because of issue related to fish size and considered that LC50 is considered to be greater than 119 mg glyphosate acid/L (highest tested dose without mortality). NOEC is set at 119 mg glyphosate acid/L (highest tested dose without effects).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Glyphosate technical
Description:	Solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9 %

2. Vehicle of test material/media: reconstituted water

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	juvenile; detailed age not stated
Size:	3.9 cm (mean), range: 3.5 – 4.4 cm
Body weight of the animals:	0.8 g (mean)
Loading:	0.4 – 0.7 g fish/L (10 fish per 15 litres of test medium)
Source:	[redacted]
Diet/Food:	None
Acclimation period:	7 days

4. Environmental conditions:

Temperature:	18.5 – 20.5°C
Photoperiod:	16 hours light / 8 hours dark (500 – 1500 lux)
pH:	3.1 – 8.5
Dissolved oxygen:	8.9 – 11.2 mg O_2/L
Conductivity:	Not stated
Hardness:	250 mg $CaCO_3$/L (reconstituted water)

5. Experimental dates of work: September 17th to September 21th 1990

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, the definitive toxicity test was performed using nominal concentrations of 59.3, 88.9, 133.3, 200 and 300 mg a.s./L dissolved in reconstituted water. Also a stability test with 300 mg a.s./L without fish was conducted. The test was conducted in a static test setup. In addition, a control group was exposed to the test medium without test substance or other additives. There was one vessel for each test concentration and one for the control group, each containing 10 fish (15 L glass containers).

Observations: Assessment of sublethal effects of after 2, 24, 48, 72 and 96 hours was conducted, while mortality was recorded daily. Temperature, pH-value and oxygen saturation of the test solutions were measured at the same time points as sublethal effects and on test initiation. Prior to the start of the test, all animals were weighed and measured. Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis using samples taken at test start and after 2, 48 and 96 h (except where the mortality was already 100%)

Statistical calculations: Descriptive statistics; the Logit-Model could not be used, since the mortality rates of 0 and 100% were within two concentrations.

II. RESULTS AND DISCUSSION

A. FINDINGS

The NOEC, LOEC and LC_{50} value are given below based on geometric mean measured concentrations.

Table B.9.2.1-22: Endpoints

Endpoints (96 h)	Glyphosate technical [mg a.s./L]
LC_{50}	between 119 and 173
LOEC	between 119 and 173
NOEC	119

Analytical data: At nominal concentrations of 88.9, 133.3 and 200 mg a.s./L, the concentration of glyphosate technical was recorded to be within the range of 80 - 120 % of nominal. At 300 mg a.s./L the concentration at test start was 79.7 % and after 2 h at 82.3 % of nominal. At the lowest test concentration (59.3 mg/L) the concentration ranged between 59.6 and 84.1 % of nominal. Therefore, the toxicity values are based on (geometric) mean measured concentrations. Analytical results are shown below.

Table B.9.2.1-23: Analytical results

Nominal concentration of glyphosate technical [mg a.s./L]	Time (hours)	Mean concentration of Samples A and B	% of nominal	Geometric mean measured concentrations [mg a.s./L]
59.3	0	44.75	75.5	43.3
59.3	2	44.65	75.3	
59.3	48	49.81	84.1	
59.3	96	35.37	59.6	
88.9	0	96.35	108.4	91
88.9	2	128.15	144.2	
88.9	48	75.10	84.5	
88.9	96	74.03	83.3	
133.3	0	120.3	90.2	119
133.3	2	123.1	92.3	
133.3	48	113.3	85.0	
133.3	96	120.0	90.0	
200	0	176.4	88.2	173
200	2	169.1	84.5	
300	0	239.1	79.7	243
300	2	146.9	82.3	

B. OBSERVATIONS

No mortality occurred at concentrations of up to 119 mg a.s./L. At the nominal concentrations of 173 and 243 mg a.s./L there was 100% mortality detected. At these high test concentrations the pH was below the critical point of 4 for *Lepomis macrochirus*. At 173 mg a.s./L sublethal effects like loss of righting reflex and an enhanced respiratory rate were observed. Supine positions at the tank bottom, affection of the motoric function, remaining at the tank bottom and an enhanced respiratory rate were notices at 243 mg a.s./L.

Table B.9.2.1-24: Effects of glyphosate technical on survival of *Lepomis macrochirus*

Nominal concentration of glyphosate technical [mg a.s./L]	**Control**	**59.3**	**88.9**	**133.3**	**200**	**300**
Geometric mean measured concentrations of glyphosate technical [mg a.s./L]	**Control**	**43.3**	**91.0**	**119**	**173**	**243**
Mortality (0h) [%]	0	0	0	0	0	0
Mortality (2 h) [%]	0	0	0	0	0	10
Mortality (24 h) [%]	0	0	0	0	90	100
Mortality (48 h) [%]	0	0	0	0	100	100
Mortality (72 h) [%]	0	0	0	0	100	100
Mortality (96 h) [%]	0	0	0	0	100	100

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The LC_{50} (96 h) for bluegill sunfish exposed to glyphosate technical ranged between 133.3 mg a.s./L and 200 mg a.s./L (nominal), corresponding to 119 mg a.s./L and 173 mg a.s./L (geometric mean measured). The 96 hour NOEC was 133.3 mg a.s./L (nominal), corresponding to 119 mg a.s./L (geometric mean measured).

This study is considered valid and the acute LC_{50} value for bluegill sunfish exposed to glyphosate technical ranged between 119 mg a.e./L and 173 mg a.e./L (geometric mean measured) and can be used in risk assessment.

Assessment and conclusion by RMS:
The pH values at the two highest nominal tested concentrations (200 and 300 mg/L) were far below the recommended range (recommended pH 6-8.5). The actual pH values were of 3.2 at 300 mg/L and 3.6 at 200 mg/L. This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The study was already considered in previous assessment (DAR/RAR).

The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options.
In Schweizer, M. et al (2019) (CA 8.2.2.1/006 see addendum of B.9 (AS) on general literature review), zebrafish (*Danio rerio*) embryos acutely exposed to glyphosate at concentrations between 1.69 and 1690.7 mg glyphosate/L for 96 hours post fertilization in buffered and unbuffered situation. A particular attention was paid on pH from 3 to 4. The study demonstrates that the severe effects detected seemed to be mainly caused by a low (glyphosate induced) pH.
Overall, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the pH should always be in a range required to maintain the health of the organisms tested (here 6.5-8.5). Therefore, as pH are below is 4 for test concentration equal or greater than 180mg/L, it can not be excluded that the high mortality observed at these concentrations was due to the acidity of the test solutions caused by high concentrations of glyphosate acid.
At 88.9 and 13.3 mg/L, pH was of 5.7 and 4.5 respectively (and no mortality was observed).

The temperature (18.5-20.5°C) was also lower than the recommended range for this species (21-25°C). It is the RMS opinion that these deviations did not significantly affect the study.

RMS notes that the actual size (35 to 44 mm) of the tested fish exceeded the recommended range (10-30 mm).

This study is considered supportive as the fish were bigger than recommended, the results can not be used for acute risk assessment. LC50 is considered to be greater than 119 mg glyphosate acid/L (highest tested dose without mortality). NOEC is set at 119 mg glyphosate acid/L (highest tested dose without effects).

Data point:	CA 8.2.1/011
Report author	[redacted]
Report year	1981
Report title	Acute Toxicity of MON 0139 (lot LURT 12011) ([redacted]-81-073) to Bluegill Sunfish (*Lepomis macrochirus*)
Report No	27201
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	Deviations from the current OECD 203 guideline (2019): *Major:* *- No analytical verification of test concentrations* *- Dissolved oxygen concentration decreased below 60% of saturation (from 9.5 mg/L to 5.5 mg/L in all tested groups: control, 100 and 1000 mg test item/L)* *Minor:* *- Fish were acclimatized for 48 hours prior to the test (7 days are required)* *- pH of the highest concentration (1000 mg test item/L) was not with the specified range of 6.0-8.5 (pH measured: 4.5 – 5.1). No pH measurements are available at 180, 320 and 560 mg/L.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Invalid

Summary

The effects of glyphosate isopropylamine salt (MON 0139) on the bluegill sunfish (*Lepomis macrochirus*) were evaluated in a 96-hour static toxicity test. Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L, corresponding to 62.5, 112, 200, 350 and 625 mg glyphosate isopropylamine salt/L (mg a.s./L) or 46.3, 83.3, 148, 259 and 463 mg glyphosate/L (mg a.e./L). In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. There was no mortality observed at any of the test concentrations up to and including 1000 mg test item/L, corresponding to 625 mg a.s./L or 463 a.e./L (nominal).

Not all validity criteria according to the OECD 203 (2019) were fulfilled since the analytical part of the study was not performed and/or reported. Taking also into account that the oxygen levels decreased below 60 %. And further minor deviations, that may affect the outcome of the study, the study is therefore considered as invalid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt (MON 0139)
Description:	Light yellow liquid
Lot/Batch #:	LURT 12011
Purity:	62.49%

2. Vehicle of test material/media and positive control: Vehicle: Soft reconstituted water
Positive control: Antimycin A

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	At least 14 days old
Size:	Length: 19 mm (mean)
Body weight:	0.14 g (mean)
Loading:	10 test individuals for 15 L test solution (= 0.09 g fish/L)
Source:	[redacted]
Diet/Food:	Daily with Standard commercial fish food (Rangen's) except 48 prior to the test
Acclimation period:	48 hours prior to the test initiation

4. Environmental conditions:

Temperature:	22 ± 1°C
Photoperiod:	16 h light
pH:	7.1
Dissolved oxygen:	9.5 mg/L
Conductivity:	Not stated
Hardness:	45 mg $CaCO_3$/L

5. Experimental dates of work: March 19th to March 23rd 1981

B. STUDY DESIGN

Experimental treatments: Based on the results of a 48-h range finding test, a definitive toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L. In addition, a control group was exposed to dilution water (soft reconstituted water) and a reference product (Antimycin A). The mortality of fish was recorded in all test concentrations and the control at 24, 48 and 96 hours. There was one vessel per treatment, containing ten fish in 5-gallon (appr. 19 L) glass vessels containing 15 L test medium.

Observations: The fish mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after the test initiation. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. The weight and length of the test fish were measured.

Statistical calculations: LC_{50} values were calculated using computer program by Stephan et al. (1978)

(A computer program for calculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.)

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification of the tested concentrations was conducted or reported.

The LC_{50} value is given below based on nominal concentrations.

Table B.9.2.1-25: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50}	>1000	>625	>463

B. OBSERVATIONS

There was no mortality observed at any of the test concentrations up to and including 1000 mg test item/L. For the reference product Antimycin A, the LC_{50} was determined to be 0.00010 mg/L. The dissolved oxygen concentration slightly dropped under 60% saturation. The pH values dropped with increasing test concentrations.

Table B.9.2.1-26: Lethal effects of glyphosate isopropylamine salt (MON 0139) to *Lepomis macrochirus*

	Control					
Test item [mg/L]	**-**	**100**	**180**	**320**	**560**	**1000**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**62.5**	**112**	**200**	**350**	**625**
Glyphosate [mg a.e./L]	**-**	**46.3**	**83.3**	**148**	**259**	**463**
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	0
Mortality (72 h) [%]	0	0	0	0	0	0
Mortality (96 h) [%]	0	0	0	0	0	0

The following validity criterion according to the OECD 203 (2019) was fulfilled:

- The control mortality was lower than 10 % at the end of the study.

The following validity criteria according to the OECD 203 (2019) were not fulfilled:

- No analytical measurement of the test concentrations was reported.
- The dissolved oxygen concentration was slightly below the trigger value of ≥60 % of the air saturation value (ranging from 9.5 to 5.5 mg/L in all tested groups: control, 100 and 1000 mg test item/L through the study).

The following points deviated from current guideline:

- Fish were acclimatised 48 hours prior to the test instead of the 7 required
- Observations occurred after 24h, 48h and 96h. The requirements are the following: a minimum of 2 observations within the first 24 hours of the study and on days 2 - 4 of the test, all vessels with living fish inspected twice per day (preferably early morning and late afternoon to best cover the 24-hour periods).
- The pH was outside of accepted range of 6.0-8.5 (pH measured: 4.9 – 5.1) in the highest concentration (1000 mg test item/L) and therefore the stock solution should have been adjusted. No pH measurements are available at 180, 320 and 560 mg/L.

These deviations may affect the outcome of the study, so the validity of the study is questionable.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a static acute fish toxicity test, the LC_{50} (96 h) for bluegill sunfish (*Lepomis macrochirus*) exposed to glyphosate isopropylamine salt (MON 0139) was determined to be >1000 mg test item/L, corresponding to >625 mg a.s./L or >463 mg a.e./L (nominal).

Not all validity criteria according to the OECD 203 (2019) were fulfilled since the analytical part of the study was not performed and/or reported. Taking also into account that the oxygen levels decreased below 60 % and further minor deviations that may affect the outcome of the study, the study is therefore considered as invalid.

Assessment and conclusion by RMS:

Major deviations from the current OECD 203 guideline (2019) were highlighted in this study.

This study is considered invalid.

Data point:	CA 8.2.1/012
Report author	[redacted]
Report year	1978
Report title	Acute Toxicity of Technical Glyphosate to Bluegill Sunfish (*Lepomis macrochirus*)
Report No	[redacted] 78-123
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 203 guideline (2019):* *Major:* *- No analytical verification of test concentrations.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Supportive

Summary

The acute effects of glyphosate technical on bluegill sunfish (*Lepomis macrochirus*) in a 96-hour static toxicity test. A definitive toxicity test was performed with glyphosate technical at nominal concentrations of 28, 42, 56, 75, 100, 120, 140 and 180 mg glyphosate technical/L. A control group was exposed to deionised water and a reference treatment group exposed to Antimycin A were also tested.

In the definitive test, the mortality of fish was recorded at 24, 48 and 96 hours after test initiation. After 96 hours of exposure to glyphosate technical, there was 100% mortality recorded in the 140 and 180 mg a.s./L treatment groups. In the 120 mg a.s./L treatment group, there was 50% mortality recorded, with no mortality recorded at or below nominal concentrations of 100 mg a.s./L. The LC_{50} (96 h) was determined to be 120 mg a.s./L (nominal concentration of glyphosate technical).

According to the current OECD 203 test guideline, despite the control validity criteria of <10% mortality being achieved, the validity of the present study according to OECD guideline 203 is questionable, since the analytical part of the study was not performed and/or reported. The study is considered supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	Not stated
Lot/Batch #:	Not stated
Purity:	Technical grade (stated)

2. Vehicle of test material/media and positive control: Vehicle: Deionised water
Positive control: Antimycin A

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Not stated
Size:	Length: 3.42 cm (mean)
Body weight:	0.96 g (mean)
Loading:	10 individuals test per vessel (19 L glass vessels) in 15 L test solution (0.64 g fish/L)
Source:	[redacted]
Diet/Food:	Daily with Standard commercial fish food (Rangen's No. 1 Fry) except 48 prior to the test
Acclimation period:	48 hours prior to the test initiation

4. Environmental conditions:

Temperature:	21 ± 1°C
Photoperiod:	Not stated
pH:	6.8 – 7.0
Dissolved oxygen:	6.2 – 8.2 mg/L
Conductivity:	Not stated
Hardness:	46 mg $CaCO_3$/L

5. Experimental dates of work: Test start: February 10th 1978

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, definitive toxicity test was

performed with glyphosate technical at nominal concentrations of 28, 42, 56, 75, 100, 120, 140 and 180 mg a.s./L in a static test setup. The test item was dissolved directly into deionised water. A control group was also prepared using fish exposed to deionised water only (soft reconstituted water).
A reference toxicant test was conducted in parallel using Antimycin A at rates between 0.024 and 0.21 mg/L, with acetone used to prepare the reference toxicant group treatment media.
A single replicate vessel was prepared per treatment, control and reference toxicant group.

Observations: Mortality was recorded in all test concentrations and the control 24, 48 and 96 hours after test initiation in the glyphosate exposure test and additionally at 72 hours in the reference toxicant test. Temperature, pH-value and oxygen saturation of the test solutions were measured on each observation date. Hardness of the test water was measured at the start of the test. Weight and length of the test fish were equally measured.

Statistical calculations: LC_{50} values were calculated along with the 95% confidence limits using Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values are given below based on nominal concentrations.

Table B.9.2.1-27: Endpoints

Endpoints (96 h)	Glyphosate [mg a.s./L]
LC_{50} (95% C.I.)	120 (111 - 130)

B. OBSERVATIONS

At and above the nominal concentration of 140 mg test item/L, 100% mortality was observed 96 hours after test initiation. At the nominal concentration of 120 mg test item/L, 50% mortality was recorded whereas no mortality was observed at and below the nominal concentration of 100 mg test item/L. For the highest concentration of reference product Antimycin A (0.021 mg/L), 70% mortality was observed 24 hours after the test initiation and no fish survived 48 hours after test initiation.

Table B.9.2.1-28: Lethal effects of glyphosate technical to *Lepomis macrochirus*

Glyphosate [mg a.s./L]	C	28	42	56	75	100	120	140	180
Mortality (24 h) [%]	0	0	0	0	0	0	30	100	100
Mortality (48 h) [%]	0	0	0	0	0	0	40	100	100
Mortality (72 h) [%]	0	0	0	0	0	0	50	100	100
Mortality (96 h) [%]	0	0	0	0	0	0	50	100	100

C = Control

III. CONCLUSIONS

Assessment and conclusion

Assessment and conclusion by applicant:

In a static acute fish toxicity test, the LC_{50} (96 h) for bluegill sunfish (*Lepomis macrochirus*) exposed to the test item glyphosate was determined to be 120 mg a.s./L (nominal).

According to the current OECD 203 test guideline, despite the control validity criteria of <10% mortality being achieved, there was no chemical analysis performed to confirm glyphosate concentration in the test media. The study is therefore not be considered valid against the current criteria.

Assessment and conclusion by RMS:

No analytical verification of test concentrations are available. For this reason, this study should be considered unreliable. However despite the absence of analytical verification, a strong effect is observed. RMS considers the results not robust enough to derive a reliable endpoint to be used for risk assessment. However an effect of the test item is obvious and it can reasonably be hypothesized that the actual LC50 lies between 100 and 140 mg glyphosate/L. The NOEC is set at 100 mg glyphosate/L.

RMS also notes that the overall size (34.2 mm) of the tested fish slightly exceeded the recommended range (10-30 mm). It is the RMS opinion that this deviation did not significantly affect the study.

This study is only informative.

Data point:	CA 8.2.1/013
Report author	[redacted]
Report year	2006
Report title	Glyphosate Technical: Acute Toxicity to Common Carp (*Cyprinus carpio*)
Report No	2060/015
Document No	-
Guidelines followed in study	OECD Guideline 203 (1992); JMAFF Testing Guideline for Toxicology Studies, 12 NohSan No. 8147, Guideline 2-7-1(2000)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation compared with OECD 203 – none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes

Acceptability/Reliability (RMS):	Valid

Summary
The effects of glyphosate acid to common carp (*Cyprinus carpio*) were evaluated in a 96-hour semi-static toxicity test (48 hour renewal of test media) conducted as limit test at a nominal test concentration of 100 mg a.s./L. A negative control (dechlorinated tap water) was prepared in parallel. Duplicate control and test vessels were prepared, each containing seven fish.
All fish were observed for sub-lethal effects and mortality at 3, 6, 24, 48, 72 and 96 hours after the start of the test (fish addition). Dissolved oxygen, pH and temperature were measured and recorded daily in each test vessel. Glyphosate acid concentrations were measured at 0, 24 and 96 hours. Glyphosate acid was not detected in the control group. Mean measured concentrations ranged from 90 to 98% of nominal concentrations.
No mortality or sub-lethal effects to common carp (*Cyprinus carpio*) were observed, when exposed to glyphosate acid at the nominal concentration of 100 mg a.s./L. All validity criteria according to the guideline OECD 203 were fulfilled.
Glyphosate acid resulted in no mortality or sub-lethal effects in common carp at 100 mg a.s./L. The 96 h LC_{50} value for common carp exposed to glyphosate acid was determined to be > 100 mg a.s./L, the highest concentration tested. The NOEC was 100 mg glyphosate acid/L. This study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7%

2. Vehicle of test material/media and positive control:
Vehicle: Dechlorinated tap water
Positive control: Pentachlorophenol sodium salt (tested in a different study)

3. Test organism:

Species:	Common carp (*Cyprinus carpio*)
Age:	Juvenile
Size:	4.2 ± 0.1 cm
Body weight:	2.05 ± 0.13 g
Loading:	0.72 g body weight/L test solution
Source:	[redacted]
Diet/Food:	no feeding during the total test period
Acclimation period:	12 days at test conditions

4. Environmental conditions:

Temperature:	20.6 – 21.2°C
Photoperiod:	16 hours light / 8 hours dark, with 20 minutes dawn and dusk transition
pH:	7.4 – 8.3 (control), 6.3 – 8.0 (treatment)

Dissolved oxygen: 8.1 - 8.8 mg/L (91 - 99% saturation at 20.6 – 21.2°C)
Conductivity: 359 – 610 µS/cm
Hardness: Approx. 100 mg $CaCO_3$/L.

5. Dates of experimental work: 2005-05-31 to 2005-06-04

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, a final toxicity test was performed under semi-static test design as limit test using a single nominal concentration of glyphosate acid of 100 mg a.s./L. The control and test media at 100 mg a.s./L were renewed at 48 hours. A negative control group (derchlorinated water) was also prepared in parallel. There were duplicate glass vessels for the test concentration and control, each containing seven test fish in 20 L test medium.

Observations: All fish were observed for sub-lethal effects and mortality after 3, 6, 24, 48, 72 and 96 hours after test initiation (fish addition). Test solutions were renewed after 48 hours. Water temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Water hardness was measured in fresh media only. Samples of fresh media were taken at o hours and samples of old test media were taken at 24 and 96 hours to be analysed for glyphosate using a HPLC method of analysis.

Statistical calculations: Since the mortality was <50 %, no statistical calculation of LC_{50} values was possible. Therefore, NOEC and LC_{50} were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Mean measured test item concentrations ranged from 90% to 98% of nominal test concentration. Therefore, endpoints were evaluated using nominal test item concentrations.

Table B.9.2.1-29: Analytical results

Sample	Nominal concentration of glyphosate acid [mg a.s./L]	Measured concentration glyphosate acid [mg a.s./L]	% of nominal
0 h (fresh media)	control	<LOQ	-
	100	95.2	95
	100	97.8	98
24 h (old media)	control	<LOQ	-
	100	90.3	90
	100	92.9	93
96 h (old media)	control	<LOQ	-
	100	98.1	98
	100	98.4	98

LOQ= Limit of quantification (5.3 mg/L)

The 96 h LC_{50} and corresponding NOEC values based on nominal concentrations are given below.

Table B.9.2.1-30: Endpoints

Endpoints (96 h)	Glyphosate acid [mg a.s./L]
LC_{50}	>100
NOEC	100

Reference test: The 96 h LC_{50} for the reference item pentachlorophenol was 0.26 mg/L, which is within the normal range of the reference material. The reference item was tested in a separate study.

B. OBSERVATIONS

During the acclimation the fish were fed with ZM Large Granule Feed as opposed to Commercial Car Pellets as this feed type was considered to be more suitable for the size of the fish. This deviation did not have any negative impact on the study validity.
At the 100 mg a.s./L concentration, there was no mortality during the 96 hours of exposure to glyphosate acid. In addition, no sub-lethal effects were observed.
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusion

Assessment and conclusion by applicant:
The 96 h LC_{50} for common carp (*Cyprinus carpio*) exposed to glyphosate acid in a limit test was determined to be >100 mg a.s./L, with a 96 hour NOEC of 100 mg glyphosate a.s./L.

This study is considered valid and the acute LC_{50} value for common carp exposed to glyphosate acid was determined to be >100 mg a.s./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
This study is considered valid.
No mortality and no sub-lethal effects have been observed at the limit dose tested of 100 mg a.s./L.
Acute LC_{50} value for common carp exposed to glyphosate acid was determined to be >100 mg glyphosate acid/L (nominal).
NOEC = 100 mg glyphosate acid/L

Data point	CA 8.2.1/014
Report author	[redacted]
Report year	1973
Report title	Information not available
Report No	95-00015
Document No	-

Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations:	Toxicity of technical glyphosate (purity> 94%) to aquatic organisms (*Cyprinus carpio*) in a 96 hours static test
Short description of results:	LC_{50} = 115 mg a.e./L
Reasons for why the study is not considered relevant/reliable or not considered as key study:	The full study report is not available to the applicant. However these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR (2015).
Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL

Assessment and conclusion by RMS:
RMS notes that this study was found in the previous RAR 2015.
This study was requested to the BVL but could not be provided to RMS.
For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical. The endpoint measured in this study is not critical and therefore its absence has no consequence on the risk assessment.

Data point:	CA 8.2.1/015
Report author	[redacted]
Report year	2000
Report title	Acute Toxicity of Glifosate Técnico [redacted] to Zebrafish (*Brachydanio rerio*)
Report No	[redacted]-D61.47/99
Document No	-
Guidelines followed in study	OECD Guideline 203 (1993)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation compared with OECD 203 – none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary

The acute effects of glyphosate technical on zebra fish (*Brachydanio rerio* – also known as *Danio rerio*) were evaluated in a 96-hour semi-static toxicity test (48 hour media renewal) conducted at nominal test concentrations of 10, 32, 56, 100, 180, 320 mg a.s./L. A control (reconstituted water) was also prepared. Vessels were prepared in duplicate with ten fish per vessel.

Observations for fish mortality and sub-lethal effects were performed at 3, 24, 48, 72 and 96 hours after the start of the test (fish addition). Dissolved oxygen, pH and temperature were measured and recorded daily in each test vessel. Glyphosate technical concentrations were measured in new and old control and test media on each day of the test. Glyphosate technical was not detected in the control group. Overall mean measured concentrations of glyphosate techncial ranged between 95.9 and 108.8 % of nominal concentrations.

During the 96-hour exposure period to glyphosate technical, at nominal concentrations up to 56 mg a.s./L, there were no sub-lethal effects or mortality recorded. At the concentration of 100 mg a.s./L, there was 15% mortality with hyperactivity observed in test fish at 48 hours onwards. At a concentration of 180 mg a.s./L and above, there was 100% mortality observed after 24 hours.

The authors concluded that the 96 hour LC_{50} for zebra fish exposed to glyphosate technical was determined to be 122.91 mg a.s./L (nominal) with a 95% confidence interval of 111.97 to 134.92 mg a.s./L. The 96-hour NOEC was 56 mg a.s./L (nominal concentrations of glyphosate technical).

RMS considers this study as supportive. In this study, 50% effects on mortality have been recorded for a nominal concentration of 123 mg glyphosate acid. The lowest nominal concentration without effect on mortality was 56 mg glyphosate acid/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate Tecnico [redacted]
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	95.0% a.s. (nominal), 95.49% a.s.(analysed)

2. Vehicle of test material/media and positive control:

Vehicle: Reconstituted water

Positive control: Potassium dichromate ($K_2Cr_2O_7$)

3. Test organism:

Species:	Zebra fish (*Brachydanio rerio)*
Age:	Not stated
Size:	Not stated
Body weight of the animals:	0.191 -0.239 g
Loading:	(0.38 to 1.44 g fish/L) 10 specimens exposed in 3 L test solution
Source:	In-house culture, previously obtained from the commercial supplier [redacted]
Diet/Food:	no feeding during the total test period
Acclimation period:	72 h (to dilution water) prior to the test initiation (no feeding 24 h prior to test start and during the test)

4. Environmental conditions:

Temperature:	24.1 – 24.5°C
Photoperiod:	16 hours
pH:	Control (start – 96 h): 7.4 – 7.5 10 mg/L (start – 96 h): 7.3 – 7.1 32 mg/L (start – 96 h): 7.0 – 6.6 56 mg/L(start – 96 h): 6.5 – 5.3 100 mg/L (start – 96 h): 5.1– 4.8 180 mg/L (start – 24 h): 4.1 – 4.0 320 mg/L (start – 24 h): 3.5 – 3.6
Dissolved oxygen:	4.9 – 5.8 mg O_2/L (61.72% - 73.06% of saturation value at 24.5°C)
Conductivity:	691 - 711 µS/cm
Hardness:	229.7 – 249.9 mg $CaCO_3$/L.

5. Dates of experimental work: 18th October to 22nd October 1999

B. STUDY DESIGN

Experimental treatments: Based on the results of a range finding test, a definitive toxicity test was performed with glyphosate technical at nominal concentrations of 10, 32, 56, 100, 180, 320 mg a.s./L in a semi-static test setup, with test media renewal after 48 hours. A negative control (reconstituted water only) was also prepared. There were two vessels per treatment, containing ten fish each (4000 mL glass vessels containing 3000 mL test medium).

Observations: All fish were observed for sublethal effects and mortality after 3, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Weight measurements were conducted of each individual fish at test initiation. Samples of test media were analysed using HPLC analysis at test initiation and after 48 and 96 hours.

Analytical procedures: Aliquots of exposure concentrations were collected at each test solution renewal. The active ingredient was analysed by Liquid Chromatography HP 1050.

Statistical calculations: LC_{50} values, along with respective 95% confidence limits were calculated using the Trimmed Spearman-Karber Method. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Mean measured concentrations of glyphosate acid ranged between 95.5% and 108.8% of the nominal test concentrations. As values were between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal test item concentration.

Table B.9.2.1-31: Analytical results

Nominal concentration of glyphosate technical [mg a.s./L]	Mean measured concentration of glyphosate technical [mg a.s./L]	% of nominal
Dilution water control	0	-
10	10.83	108.3
32	33.28	104.0
56	58.37	104.2
100	108.80	108.8
180	171.96	95.5
320	346.34	108.2

The 96 h LC_{50} and corresponding NOEC values based on nominal concentrations are given below.

Table B.9.2.1-32: Endpoints

Endpoints (96 h)	Glyphosate technical [mg a.s./L]
LC_{50} (95% CI)	122.91 (111.97 – 134.92)
NOEC	56

CI= Confidence interval

B. OBSERVATIONS

At the 180 mg a.s./L concentrations and higher, 100% mortality was observed after 24 hours exposure to glyphosate technical. At 100 mg a.s./L, there was 20% mortality after 72 hours and 30% mortality after 96 hours, with hyperactivity observed in test fish at 48 hours onwards. At 56 mg a.s./L and lower, no fish mortalities or sub-lethal effects were observed throughout the test period.
The biological observations recorded during the test are presented in the table below.

Table B.9.2.1-33: Lethal effects of glyphosate acid to zebra fish

Nominal concentration of glyphosate technical [mg a.s./L]	Number of dead fish and observed symptoms				
	3 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
10	0	0	0	0	0
32	0	0	0	0	0
56	0	0	0	0	0
100	0	0	0 HA	2 HA	3 HA
180	0 LE	10	10	10	10
320	9 LE	10	10	10	10

[1] Dead fish are added to the sum of fish with symptoms
* All fish dead
LE: loss of equilibrium
HA: hyperactivity

The 96 h LC_{50} (95% CL) for the reference product was calculated to be 79.54 (68.87 – 91.88) mg/L based on nominal concentrations.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 96 hour LC_{50} for zebra fish (*Brachydanio rerio*) exposed to glyphosate technical was 123 mg a.s./L (nominal) with a 95% confidence interval of 111.97 to 134.92 mg a.s./L. All validity criteria according to OECD 203 were fulfilled. The 96-hour NOEC was 56 mg a.s./L (nominal concentration of glyphosate technical).

Since the analytical methods and substance verification were not documented in detail the study is therefore considered as supportive for the risk assessment.

Assessment and conclusion by RMS:

The pH values at the three highest tested concentrations (100,180 and 320 mg/L) were below the recommended range (recommended pH 6-8.5). This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options. Overall, the high mortality observed at these concentrations can be explained by the acidity of the test solutions (at least partly).

Analytical verifications were not documented in detail as highlighted above by the applicant. The following table was extracted from the raw data. Note that the last line of the table corresponds to the stock solution (1000 mg/L).

concentração nominal	0 - 24 h	24 - 48 h	48 - 72 h	72 - 96 h	media conc. efetiva	%
controle	0	0	0	0	0	0
10	10,54	9,68	11,58	11,52	10,83	108,
32	31,36	33,95	34,52	–	33,28	104,
56	59,83	59,47	55,82	–	58,37	104,2
100	106,85	104,63	114,94	–	108,80	108,8
180	171,96	–	–	–	171,96	95,5
320	346,34	–	–	–	346,34	108,2
1000	1064,31	1047,06	1083,25	—	1064,87	106,4

It is agreed by RMS that analytical verifications are desirable throughout the test particularly at a concentration around the expected LC50. However for semi-static renewal tests, the OECD guideline recommends to perform this verification "at least twice over one exposure period (before and after renewal of test solutions". This was done for most of the tested concentrations but only in the renewal test solution.

All other validity criteria according to OECD 203 were fulfilled.
RMS considers this study as supportive. In this study, 50% effects on mortality have been recorded for a nominal concentration of 123 mg glyphosate acid. The lowest nominal concentration without effect on mortality was 56 mg glyphosate acid/L.

Data point:	CA 8.2.1/016
Report author	[redacted]
Report year	1993
Report title	Acute Toxicity Testing in Fish, Test Article: 'Glyphosate isopropylamine salt'
Report No	80-91-2328-02-93
Document No	-
Guidelines followed in study	OECD Guideline 203; EEC Directive 92/69
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations to OECD 203 (2019):* *Major:* *- None.* *Minor:* *- Test species: Leuciscus idus* *- Loading rate: slightly above 1 g fish/L (1.065 g fish/L)*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
The effects of glyphosate isopropylamine salt on golden orfe (*Leuciscus idus*) were evaluated in a 96-hour static toxicity test. The toxicity test was performed using nominal concentrations of 498, 887, 1578, 2809 and 5000 mg test item/L, corresponding to 307, 546, 972, 1730 and 3080 mg glyphosate isopropylamine salt/L (mg a.s./L) or 227, 405, 720, 1282 and 2282 mg glyphosate/L (mg a.e./L). Further a dechlorinated and deionised tap water control was used. Ten fish were exposed to each treatment level. Mortality was recorded after 2, 4, 24, 48, 72 and 96 hours after the start of the test. Records on visible abnormalities were equally made. At termination of the test, all animals were weighed and measured.
Analytical control measurements of the actual concentrations of the test item were performed by mean of HPLC analysis. Glyphosate isopropylamine salt levels were determined based on the concentrations of glyphosate. Three representative concentrations (498, 1578 and 5000 mg test item/L, corresponding to 307, 972 and 3080 mg a.s./L or 227, 720 and 2282 mg a.e./L) were analysed at 24 h intervals.
At and below the nominal concentration of 5000 mg test item/L, no mortality was observed during the exposure period. In comparison to the control group, no abnormal effects were seen at or below the highest concentration tested. All validity criteria according to the guideline OECD 203 were fulfilled.

In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for golden orfe (*Leuciscus idus*) was determined to be > 5000 mg test item/L, corresponding to 3080 mg glyphosate isopropylamine salt/L (mg a.s./L) or 2282 mg glyphosate/L (mg a.e./L) (nominal). The NOEC was determined to be ≥5000 mg test item/L, corresponding to ≥3080 mg glyphosate isopropylamine salt/L or ≥ 2282 mg glyphosate (mg a.e./L) (nominal).
The applicant considered this study valid.
RMS considered that this study should be considered as supportive as the sensitivity of the tested species is unknown in particular for the individuals of that size (5.90 cm, mean length).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C

2. Vehicle of test material/media: dechlorinated deionised tap water

3. Test organism:

Species:	Golden orfe (*Leuciscus idus*)
Age:	not stated
Size and Weight:	5.90 cm (mean length of 10 representative individuals), 2.13 g mean body weight
Loading:	10 L for 5 fish (1.065 g fish/L)
Source:	[redacted]
Diet/Food:	no feeding during test
Acclimation period:	≥ 48 h in a 250 L glass aquarium under general test conditions
Body weight of the animals:	2.13 g (mean body weight of all individuals)

4. Environmental conditions:

Temperature:	18.8-21.6°C
Photoperiod:	16 hours light / 8 hours dark, 600 – 800 lux
pH:	7.5 – 8.5
Dissolved oxygen:	> 60% of air saturation (approx. 6.0 mg O_2/L)
Conductivity:	not stated
Hardness:	14° dH (1dH= 10 mg CaO/L)

5. Experimental dates of work: 03rd September to 19th September 1993

B. STUDY DESIGN

Experimental treatments

Based on the results of a range finding test, the definitive toxicity test was performed using nominal concentrations of 498, 887, 1578, 2809 and 5000 mg test item/L in a static test setup. In addition, a control group was exposed to dechlorinated and deionised tap water only. There were two vessels per treatment, each containing five fish (12 L glass containers containing 10 L test medium).

Observations

Assessment of effects and mortality of test fish after 2-4, 24, 48, 72 and 96 hours was conducted. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Hardness of the test water was measured at the start of the test. Mortality was recorded on each observation date. Records on visible abnormalities were equally made. At start and termination of the test, all animals were weighed and measured. Analytical control measurements of the actual concentrations of the test item were performed by mean of HPLC analysis. Glyphosate isopropylamine salt levels were determined based on the concentrations of glyphosate. Three representative

concentrations (498, 1578 and 5000 mg test item/L, corresponding to 307, 972 and 3080 mg a.s./L or 227, 720 and 2282 mg a.e./L) were analysed at 24 h intervals.

Statistical calculations: Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values are given below based on nominal concentrations.

Table B.9.2.1-34: Endpoints

Endpoints (96 h)	Test item [mg/L]	Glyphosate isopropylamine salt [mg a.s./L]	Glyphosate [mg a.e./L]
LC_{50}	>5000	>3080	>2282
NOEC	5000	3080	2282
LOEC	5000	3080	2282

Analytical data: Analytical control measurements were performed on three representative concentration levels of glyphosate isopropylamine salt, at 498, 1578 and 5000 mg test item/L. Before introduction of the fish 81.8%, 94.6% and 96.2% of glyphosate were recovered at 498, 1578 and 5000 mg test item/L, respectively. In the aged test media 85.3%, 103.9% and 90.8% of the nominal concentration were recovered. Consequently, during the test period of 96 hours the fish were exposed to a mean concentration of 93.3% (average for test concentrations of 498, 1578 and 5000 mg test item/L, respectively) of nominal concentration.

As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.1-35: Analytical results

Nominal concentration of the test item [mg/L]	Nominal concentration of glyphosate [mg a.e./L]	Time (hours)	Measured concentration of glyphosate [mg a.e./L]	% of nominal
498	227	0	186	81.8
		48	194	85.5
		96	194	85.3
1578	720	0	681	94.6
		48	665	92.4
		96	748	103.9
5000	2282	0	2196	96.2
		48	2215	97.1
		96	2072	90.8

B. OBSERVATIONS

Clinical observations:

At or below the nominal concentration of 5000 mg test item/L, no mortality was observed during the exposure period.

In comparison to the control group, no abnormal effects were seen at or below the concentration of 5000 mg test item/L.

Table B.9.2.1-36: Lethal effects of glyphosate isopropylamine salt to golden orfe

	Control					
Test item [mg/L]	**-**	**498**	**887**	**1578**	**2809**	**5000**
Glyphosate isopropylamine salt [mg a.s./L]	**-**	**307**	**546**	**972**	**1730**	**3080**
Glyphosate [mg a.e./L]	**-**	**227**	**405**	**720**	**1282**	**2282**
Mortality (2-4 h) [%]	0	0	0	0	0	0
Mortality (24 h) [%]	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	0
Mortality (72 h) [%]	0	0	0	0	0	0
Mortality (96 h) [%]	0	0	0	0	0	0

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusion

Assessment and conclusion by applicant:
In a static acute toxicity study of glyphosate isopropylamine salt, the LC_{50} (96 h) for golden orfe (*Leuciscus idus*) was determined to be > 5000 mg test item/L, corresponding to 3080 mg glyphosate isopropylamine salt/L (mg a.s./L) or 2282 mg glyphosate/L (mg a.e./L) (nominal). The NOEC was determined to be ≥5000 mg test item/L, corresponding to ≥3080 mg glyphosate isopropylamine salt/L or ≥ 2282 mg glyphosate (mg a.e./L) (nominal).

This study is considered valid and the acute LC_{50} value for golden orfe exposed to glyphosate isopropylamine salt was determined to be >2282 mg a.e./L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
This study is considered valid according to validity criteria of OECD 203.

This data should be considered as additional data as the tested species, the golden orfe (*Leuciscus idus*) is not listed in the recommended species of OECD 203 and its sensitivity, and more particularly the individuals of that size (5.90 cm, mean length), is not known. Given the size the individuals can be considered as juvenile.

Acute LC50 value for golden orfe exposed to glyphosate isopropylamine salt >2282 mg a.e./L (nominal) and NOEC = 2282 mg a.e./L (supportive)

Data point:	CA 8.2.1/017
Report author	[redacted]
Report year	1998
Report title	96-Hour Acute Toxicity Study in Rainbow trout with (Aminomethyl)Phosphonic Acid (Static)
Report No	232469
Document No	-
Guidelines followed in study	EEC directive 92/69, Part C.1 OECD guidelines No. 203 (1992).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation compared with OECD 203 – none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The toxicity of AMPA (Aminomethyl- phosphonic acid) on rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour static toxicity test conducted as a limit test at a nominal test concentration of 100 mg/L. A negative control (dilution water only) was prepared in parallel. Seven fish were added to the control and each AMPA treated vessel.

Observations for sub-lethal effects and mortality were performed at 2, 24, 48, 72 and 96 hours after the start of the test (fish addition). Dissolved oxygen, pH and temperature were measured and recorded daily in each test chamber. AMPA concentrations were measured at 0 (freshly prepared test media before fish addition) and 96 hours (test end). AMPA was not detected in the control group. Mean measured concentrations ranged between 97 to 105% of nominal concentrations. Toxicity was evaluated based on the nominal concentrations.

There were no sub-lethal effects or fish mortality observed at the nominal 100 mg/L concentration during the 96 h exposure to AMPA. All validity criteria according to the guideline OECD 203 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Aminomethyl - phosphonic acid (AMPA)
Description:	White powder
Lot/Batch #:	A010047101
Purity:	99%

2. Vehicle of test material/media and positive control: Vehicle: Tap water

Positive control: Pentachlorophenol

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juveniles
Size:	4.14 ± 0.34 cm
Body weight of the animals:	0.54 ± 0.20 g (mean weight of 10 representative individuals)
Loading:	0.38 g fish/litre (7 fish per 10 litres of test medium)
Source:	[redacted]
Diet/Food:	Last feeding at about 30 hours prior to the test and no feeding during the total test period
Acclimation period:	At least 12 days after delivery

4. Environmental conditions:

Temperature:	14.2 – 14.8°C
Photoperiod:	16 hours light / 8 hours dark
pH:	7.3 – .8.4
Dissolved oxygen:	9.3 – 9.7 mg O_2/L
Conductivity:	Not stated
Hardness:	2.4 mmol/L

5. Experimental dates of work: 24th May to 29th 1998

B. STUDY DESIGN

Experimental treatments: The test was conducted as a static (without renewal) 96 hour limit test at a nominal test concentration of 100 mg/L of AMPA, based on the results of a range finding test. The test media was prepared by direct addition of AMPA to tap water. A negative control (dilution water) was prepared in parallel. Single vessels (18-L glass aquariums) containing 10 litres of control, or test media were prepared. Seven fish were added to each vessel at the start of the test.

Observations: All fish were observed for sub-lethal effects and mortalities after 2, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis. Hardness of the test water was measured at test initiation only.

Prior to the start of the test, ten representative fish from the fish stock used in the test were weighed (wet weight (g)) and measured (total length (cm)).

Samples of control or test media were taken at test start (0 hours) before fish addition and at 96 hours (test end). Concentrations of AMPA in each sample were determined using an HPLC method of analysis.

Statistical calculations: Since the mortality was < 50%, no statistical calculation of LC_{50} values was possible. Therefore, NOEC and LC_{50} were determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

Analytical data: Measured concentrations of AMPA in media samples taken at the start of the test before fish introduction were 105% of nominal. At the end of the test, concentrations in the aged test media were 97% of nominal.

Table B.9.2.1-37: Analytical results

Nominal concentration of AMPA [mg/L]	Time [hours]	Measured concentration of AMPA [mg/L]	% of nominal
water control	0	n.d.	-
100	0	105	105
water control	96	n.d.	-
100	96	96.7	97

n.d. = not determined

The mean measured concentration of AMPA ranged between 80 and 120% of nominal, therefore the ecotoxicological endpoints were evaluated based on the nominal AMPA concentrations.

The 96 hour LC_{50} and NOEC values for rainbow trout exposed to AMPA are given below.

Table B.9.2.1-38: Endpoints

Endpoints (96 h)	Aminomethyl -phosphonic acid (AMPA) [mg/L]
LC_{50}	>100
NOEC	100

Reference test: The determined 96 h-LC_{50} for the reference item pentachlorophenol was 0.30 mg/L, which correspond well with the historical range of 0.10 - 0.46 mg/L. Thus, the sensitivity of trout from the present batch corresponded with the historical data.

B. OBSERVATIONS

There were no sub-lethal effects or mortality observed in fish exposed to AMPA during the 96 hours limit test at 100 mg/L.

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusion by applicant:
Under the conditions of the present test AMPA induced no visible effects in rainbow trout at 100 mg/L (nominal). The 96 h LC_{50} for rainbow trout exposed to AMPA was determined to be >100 mg/L (nominal). The 96 hour NOEC for rainbow trout exposed to AMPA was considered to be ≥100 mg/L (nominal), the maximum concentration tested.

This study is considered valid and the acute LC_{50} value for rainbow trout exposed AMPA was determined to be >100 mg/L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
This study is considered valid.
Acute LC50 value for rainbow trout exposed to AMPA >100 mg/L (nominal).

Data point	CA 8.2.1/018
Report author	Anonymous
Report year	1994
Report title	No information available
Report No	94-00499
Document No	-
Guidelines followed in study	Information mentioned in the Monograph 2001: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Information mentioned in the Monograph: The data presented below were generated in accordance with […] the appropriate GLP-requirements.
Previous evaluation	According to the applicant: Yes, accepted in RAR (2015).
Short description of study design and observations	Acute toxicity of the metabolite aminomethyl phophenic acid (AMPA) to Rainbow trout (*Oncorhynchus mykiss*) static test, 96 hours.
Short description of results	Test item: AMPA LC_{50} 96 h >180 mg/L NOEC 96 h > 8 mg/L
Reasons for why the study is not considered relevant/reliable or not considered as key study	The full study report is not available to the applicant. However, these data were provided in the Monograph 2001. Study was considered as valid in the Monograph 2001 but it was not mentioned in the RAR 2015. The study is therefore not considered valid.
Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS:
RMS notes that this study was not found in the previous RAR 2015.
This study was requested to the BVL but could not be provided to RMS.
For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical. The endpoint measured in this study is not critical and therefore its absence has no consequence on the risk assessment.

Data point:	CA 8.2.1/019
Report author	[redacted]
Report year	1991
Report title	Acute Toxicity of AMPA to Rainbow Trout (*Oncorhynchus mykiss*)
Report No	[redacted]-90-402
Document No	-
Guidelines followed in study	OECD Guideline 203; Guideline 72-1; U.S. EPA-FIFRA, 40 CFR, Section 158.145
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation compared with OECD 203 (2019) – none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary
The effects of AMPA on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour static toxicity test. The toxicity test was performed with AMPA at nominal concentrations of 32, 56, 100, 180, 320, 560 and 1000 mg/L. In addition, a control group was exposed to dilution water (soft blended water). There was one vessel per treatment containing ten fish (19 L glass vessels containing 15 L test medium). The fish mortality, loss of equilibrium, light discoloration, dark discoloration, fish on the bottom of test chamber, surfacing, quiescence, erratic swimming, excitability and/or laboured respiration were observed in all test concentrations and the control every 24 hours until finalisation of the test (24, 48, 72 and 96 hours). Dead individuals were removed from the test vessels after each observation.
80% and 90% mortality after 96 hours was observed in the 560 and 1000 mg/L test item treatments, respectively. Laboured respiration was noted in the 56 and 100 mg/L test item treatments only at 3 hours of exposure to AMPA. No abnormal effects were noted in these two chambers after this time. All validity criteria according to the OECD guideline 203 were fulfilled.

In a static acute fish toxicity test, the LC_{50} (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to AMPA was determined to be 520 mg/L. The NOEC of 32 mg/L is based on the assessment after 3 hours, therefore the relevant NOEC at 96 h was determined to be 100 mg /L.

The applicant considered the study as valid.

RMS would have considered this study as supportive only (analytical results not available) but as no validation data for analytical method was available (see Volume 3 CA B.5). As the analytical verification is a validity criteria, the study should be considered invalid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA ((Aminomethyl)phosphonic acid)
Description:	White powder
Lot/Batch #:	HET-9001-1463T

Purity:	94.38%
2. Vehicle of test material/media:	Soft blended water
3. Test organism:	
Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	not stated
Size:	3.9 ± 0.3 cm
Body weight:	0.79 ± 0.19 g
Loading:	0.53 g/L test solution
Source:	[redacted]
Diet/Food:	none
Acclimation period:	72 h (To the test temperature) prior to the test initiation (No feeding during the acclimation period)
4. Environmental conditions:	
Temperature:	12°C
Photoperiod:	16 hours light daily, with 30 minutes transition period (110 footcandles)
pH:	4.2 - 7.6.
Dissolved oxygen:	7.1 - 9.4 mg/L (69 - 91% saturation at 12°C)
Conductivity:	130 µMhos/cm
Hardness:	40-48 mg $CaCO_3$/L.
5. Dates of experimental work:	26th to 30th October, 1990

B. STUDY DESIGN

Experimental treatments

Based on the results of a range finding test, definitive toxicity test was performed with AMPA at nominal concentrations of 32, 56, 100, 180, 320, 560 and 1000 mg/L with one vessel per treatment, each containing ten fish (19 L glass vessels containing 15 L test medium).

Observations

Mortality, loss of equilibrium, light discoloration, dark discoloration, fish on the bottom of test chamber, surfacing, quiescence, erratic swimming, excitability and/or laboured respiration were monitored in all test concentrations and the control every 24 hours for 96 hours test duration (24, 48, 72 and 96 hours). Any dead individuals were removed from the test vessels after each observation. Temperature, pH-value and oxygen saturation of the test solutions were measured on a daily basis in all test concentrations with live fish. Hardness of the test water was measured at the start of the test. Mortality was recorded on each observation date. Records on visible abnormalities were equally made. Weight and length measurements were made on the control group of fish at the termination of the test. Analytical control measurements of the actual concentrations of the test item were performed and the results are reported in a separate study (study number: [redacted]-90-403).

Statistical calculations

The LC_{50} values, along with their respective confidence limits were calculated using a computerized program developed by Stephan *et al*. (1978) (A computer program for calculating an LC_{50}. U.S. Environmental Protection Agency, Duluth, Minnesota, pre-publication manuscript, August, 1978.)

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: According to the results presented in the analytical study (study number [redacted]-90-403), mean recovery of the test item was 102 ± 1.6% of the nominal test concentrations. Therefore, the ecotoxicological endpoints were based on nominal concentrations of the test item.

According to the current requirements the 3 hours observation time point is not relevant, and therefore based on 24h and 72h observations, the NOEC can be set to 100 mg/L (data detailed in the effect table in observation part of the summary). The LC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.1-39: Endpoints

Endpoints (96 h)	**AMPA [mg/L]**
LC_{50} (95% CI)	520 (410 - 660)
NOEC	100

CI= Confidence interval

B. OBSERVATIONS

Environmental observations:

The pH decreased as the concentration of AMPA increased.

Clinical observations:

80% and 90% mortality was observed in the 560 and 1000 mg/L test concentrations after 96 hours exposure to AMPA, respectively. Laboured respiration was noted in the 56 and 100 mg/L concentrations only after 3 hours of exposure to AMPA. No abnormal effects were noted in these two chambers after this time. At or above the concentration of 320 mg/L, different abnormalities were observed and reported in the table below.

Table B.9.2.1-40: Lethal effects of AMPA to rainbow trout

	Control	**AMPA [mg/L]**						
	-	**32**	**56**	**100**	**180**	**320**	**560**	**1000**
Mortality (3h) [%]	0	0	0	0	0	0	0	0
Symptoms (3h) [%]	100N	100N	60N 40A	30N 70A	10N 90A	100A	100A	100A
Mortality (24h) [%]	0	0	0	0	0	0	0	10
Symptoms (24h) [%]	100N	100N	100N	100N	90N 10A	60N 40A	20N 80A	100A
Mortality (48h) [%]	0	0	0	0	0	0	10	10
Symptoms (48h) [%]	100N	100N	100N	100N	100N	100A	100A	100A
Mortality (72h) [%]	0	0	0	0	0	0	20	70
Symptoms (72h) [%]	100N	100N	100N	100N	90N 10A	100A	100A	100A
Mortality (96h) [%]	0	0	0	0	0	0	80	90
Symptoms (96h) [%]	100N	100N	100N	100N	100N	100A	100A	100A

N = normal; A = Affected (this could be surfacing; on bottom of test vessel, quiescent, laboured respiration and loss of equilibrium; dark discoloration)

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10% (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a static acute fish toxicity test of AMPA, the LC_{50} (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to AMPA was determined to be 520 mg/L. The NOEC (96 h) was determined to be 100 mg/L.

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to AMPA was determined to be 520 mg/L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:

Mortality was observed at the two highest concentrations (560 and 1000 mg/L). The pH values at these concentrations (4.5 and 4.2 respectively) were below the recommended range (recommended pH 6-8.5). This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of AMPA). The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-8.5, the stock solution should be adjusted to lie within the specified range of pH 6.0-8.5 (OECD, 2019). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options. Overall, the high mortality observed at these concentrations can be explained by the acidity of the test solutions (at least partly).
The study was already considered in previous assessment (DAR/RAR).

According to the study report, analytical control measurements of the actual concentrations of the test item were performed and the results are reported in a separate study (study number: [redacted]-90-403). According to the applicant and the study author, mean recovery of the test item was 102 ± 1.6% of the nominal test concentrations. The study [redacted]-90-403 was checked by RMS however analytical results were not found (table of results was missing). For this reason, RMS considers the results of this study ([redacted]-90-402) as informative only. RMS would have considered this study as supportive only (analytical results not available) but as no validation data for analytical method was available (see Volume 3 CA B.5). As the analytical verification is a validity criteria, the study should be considered invalid.

Laboured respiration was noted in the 56 and 100 mg/L concentrations only after 3 hours of exposure to AMPA (and also at higher concentrations). No abnormal effects were noted in these two chambers after this time.

The LC50 (96 h) for rainbow trout (*Oncorhynchus mykiss*) exposed to AMPA has been estimated to be 520 mg AMPA/L

This study is considered as invalid (analytical results not available, validation data for analytical method not available).

Data point:	CA 8.2.1/020
Report author	[redacted]
Report year	1993
Report title	AMPA: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*)
Report No	X582/A
Document No	
Guidelines followed in study	OECD 203
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations to OECD 203 (2019): none*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
The effects of AMPA technical (Aminomethylphosphonic acid) to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-hour static toxicity test conducted with nominal test concentrations of 18, 32, 56, 100 and 180 mg/L. Furthermore, a dilution water control was tested. Ten fish were exposed to each treatment (1 replicate per concentration).
Mortality was recorded, 24, 48, 72 and 96 hours after the start of the test. Records on visible abnormalities were equally made. Dissolved oxygen, pH and temperature were measured and recorded daily in each test chamber. Test item concentrations were verified at 0, 48 and 96 hours by HPLC. Mean measured concentrations ranged from 100 to 111% of nominal concentrations.

No mortality occurred during the 96 h exposure time (LC50 >180 mg AMPA/L (nominal)). Sub-lethal effects like dark discolouration, sounding and loss of balance were recorded starting at a concentration of 32 mg test item/L. All validity criteria according to the guideline OECD 203 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	AMPA technical (Aminomethylphosphonic acid)
Description:	Not stated
Lot/Batch #:	Not stated
Purity:	85%

2. Vehicle of test material/media: Dechlorinated, filtered tap water

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	juvenile
Size:	45 - 60 mm (mean: 50 mm)
Body weight of the animals:	1.14 – 2.82 g/ fish (mean: 1.70 g)

Loading: 0.85 g fish/L (in the dilution water control)

Source: [redacted]

Diet/Food: none

Acclimation period: 18 days

4. Environmental conditions:

Temperature: 14.2 – 15.2°C

Photoperiod: 16 hours light / 8 hours dark with a 20 minute transition period

pH: 7.22 – 7.66

Dissolved oxygen: 9.4 - 10 mg O_2/L

Conductivity: 227 µS/cm³ in the dilution water

Hardness: 41.3 mg $CaCO_3$/L

5. Dates of experimental work: 6th December to 10th December 1993

B. STUDY DESIGN

Experimental treatments

The toxicity test was performed using nominal concentrations of 18, 32, 56, 100 and 180 mg AMPA technical/L prepared using dechlorinated and filtered tap water treated with ultraviolet steriliser.
The test was conducted 96 h in a static test setup. In addition a control group was exposed to the test medium without test substance or other additives. There was one vessel per test concentration and one for the control group, each containing ten fish (27 L borosilicate glass vessel containing 20 L test medium).

Observations

Assessment of sublethal effects and mortality of test fish was conducted after 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water were controlled at test initiation.
Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis at test start and after 48 and 96 hours.

Statistical calculations: Descriptive statistic

II. RESULTS AND DISCUSSION

A. FINDINGS

The LC_{50} values and the NOEC are given below based on nominal concentrations.

Table B.9.2.1-41: Endpoints

Endpoints	AMPA technical [mg/L]
LC_{50} (24 h)	> 180
LC_{50} (48 h)	> 180
LC_{50} (72 h)	> 180
LC_{50} (96 h)	> 180
NOEC (96 h)	18

Analytical data:
The mean measured concentrations of AMPA technical ranged from 100 to 111%.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.1-42: Analytical results

Nominal concentration of AMPA technical [mg/L]	Measured concentration of AMPA technical [mg/L]			Mean measured concentration of AMPA technical [mg/L]	% of nominal
	0 h	48 h	96 h		
Control	<7.9	<7.9	<7.9	<7.9	-
18	22	21	18	20	111
32	35*	34*	32	34	106
56	58	58	56	57	102
100	110	91	98	100	100
180	190	160	180	180	100

* triplicate analyses

B. OBSERVATIONS

No mortality occurred up to the highest test AMPA technical concentration of 180 mg/L. Sub-lethal effects like dark discolouration, sounding and loss of balance were observed at 32, 100 and 180 mg/L respectively. The results of the test are depicted in the following tables.

Table B.9.2.1-43: Effects of AMPA technical to rainbow trout

Nominal concentration of AMPA technical [mg/L]	Number of dead fish / observed symptoms (% affected)			
	24 h	48 h	72 h	96 h
Control	0 / -	0 / -	0 / -	0 / -
18	0 / -	0 / -	0 / -	0 / -
32	0 / -	0 / -	0 / S, LB (11 – 30% test population)	0 / S, LB, DC (11 – 30% test population)
56	0 / -	0 / -	0 / -	0 / -
100	0 / -	0 / -	0 / S, LB (< 10% test population)	0 / S, LB (< 10% test population)
180	0 / -	0 / -	0 / S (11 – 30% test population)	0 / S, LB (> 30% test population)

S: Sounding
DC: Dark colouration
LB: Loss of balance

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

Assessment and conclusions

Assessment and conclusion by applicant:
The LC_{50} (96 h) for rainbow trout exposed to AMPA technical was >180 mg/L (nominal). The NOEC after 96 h exposure to AMPA was 18 mg/L (nominal).

This study is considered valid and the acute LC_{50} value for rainbow trout exposed to AMPA technical was >180 mg/L (nominal) and can be used in risk assessment.

Assessment and conclusion by RMS:
The batch number is not reported.
Temperature (14.2-15.2°C) slightly exceeded the recommended range (10-14°C) but this is considered as a minor deviation.
This study is valid.
Acute LC50 value for rainbow trout exposed to AMPA technical >180 mg AMPA/L (nominal)

Data point:	CA 8.2.1/021
Report author	Antunes, A. M. *et al.*
Report year	2017
Report title	Gender-specific histopathological response in guppies *Poecilia reticulata* exposed to glyphosate or its metabolite aminomethylphosphonic acid
Document No	DOI 10.1002/jat.3461
Guidelines followed in study	None
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Not applicable*
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability (RMS):	Relevant and reliable with restriction

For detailed summary of this article proposed as relevant and reliable by both applicant and RMS please refer to the appendix on Volume 3 CA B.9 on literature data related to ecotoxicologyunder point B.9.2.1.

Data point:	CA 8.2.1/022 CA 8.2.1/023
Report author	Gholami, S.J. *et al*.
Report year	2013
Report title	Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (*Cyprinus carpio*, Linnaeus, 1758)
Document No	ISSN: 2008-2525

Guidelines followed in study	OECD 203
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*None*
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability (RMS):	Relevant but reliable with restriction

For detailed summary of this article proposed as relevant and reliable by both applicant and RMS please refer to the appendix on Volume 3 CA B.9 on literature data related to ecotoxicologyunder point B.9.2.1.

B.9.2.2. Long-term and chronic toxicity to fish

B.9.2.2.1. Fish early life stage toxicity test

Data point:	CA 8.2.2.1/001
Report author	[redacted]
Report year	2010
Report title	Glyphosate acid: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
Report No	1005.029.321
Document No	
Guidelines followed in study	OECD Guideline 210 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 210 guideline (2013): none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid but not reliable for risk assessment purpose

Summary

The effects of glyphosate acid on the early life-stages of rainbow trout was determined under flow-through (continuous renewal) exposure conditions. Fertilized eggs of *Oncorhynchus mykiss* were exposed for 85 days to nominal glyphosate acid concentrations of 0.095, 0.305, 0.977, 3.125 and 10.0 mg a.s./L. Initially, 50 fertilized eggs were exposed in duplicate exposure vessels at each of the five concentrations, with duplicate negative control groups (dilution water only) run in parallel.

Eggs were fertilized in the laboratory directly before addition to egg cups and remained undisturbed in the test system in the dark until hatching success was determined on days 22 to 26, based on the number

of viable eggs. On day 26 (complete hatch), twenty fish fry per replicate i.e. 40 organisms per treatment level and control were transferred from egg cups to surrounding test media, where the development and survival was evaluated until test termination. Dissolved oxygen (DO) concentrations, pH and temperature were measured and recorded in each test vessel at experimental start and weekly thereafter until test termination (day 85). Glyphosate acid concentrations were measured on test days 0, 6, 13, 20, 27, 33, 41, 48, 55, 62, 70, 76 and 85. Glyphosate acid was not detected in the control group. Mean measured concentrations were substantially achieved and ranged between 85.7 and 96.3% of nominal concentrations. Ecotoxicological endpoint evaluation was based on overall mean measured glyphosate acid concentrations.

No statistical significant differences were detected for normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight), when compared to the control group. All validity criteria according to OECD 210 were satisfied.

In a fish early life stage study performed with rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid, the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) were determined to be = 9.63 and > 9.63 mg a.s./L, respectively, based on geometric mean measured concentrations. The applicant considered this study as valid. RMS considers the study valid but not reliable for risk assessment. (see commenting box below).

I. MATERIALS AND METHODS

A. MATERIALS

Materials and Methods

1. Test material:

Test item:	Glyphosate acid
Lot/Batch #:	GLP-0807-19475-T
Purity:	96.03%

2. Vehicle of test material/media: reconstituted well water

3. Test organism:

Species:	Rainbow trout (*Oncorhynchus mykiss*) eggs and milt
Age of eggs:	Eggs and milt were less than 36 hours old at fertilization. The time between fertilization and egg addition to test system was less than 3.5 hours
Number of animals/dose level:	40 organisms per replicate i.e. 40 organisms per treatment level and control
Supplier:	[redacted], commercial fish supplier located in [redacted]
Mean loading rate (biomass per volume of test solution)	0.31 g/L per 24 hours

4. Environmental conditions:

Temperature:	Continuously measured temperature: 9.4 to 13.1°C Single-point measured temperature: 11.3 to 13.9ºC
pH:	7.14 to 8.44

Dissolved oxygen:	> 60% ASV for study duration
Conductivity of test medium:	340 to 450 µS/cm
Hardness of test medium:	153 to 184 mg/L $CaCO_3$
Photoperiod:	16 hours with a 30 minute transition from Day 32 until test completion. Light intensity was 137 to 377 lux.
	Eggs and larvae were shielded from all light during the incubation and hatching phases until one week after hatching
5. Dates of experimental work:	May 14th to August 10th 2009

B. STUDY DESIGN

Experimental treatments

The fish early life-stage toxicity test was performed under flow-through exposure conditions, using a constant-flow test item delivery system, supplying the appropriate test medium to duplicate exposure vessels at each of the five concentrations and the duplicate negative dilution water control vessels. The fertilized eggs were exposed to glyphosate acid at test concentrations of 0.095, 0.305, 0.977, 3.125 and 10.0 mg a.s./L for 85 days.

Twelve impartially located exposure vessels were maintained in a temperature-controlled water bath designed to maintain the test solution temperatures at 12 ± 2°C. During the egg exposure phase and until one week after hatching the test area was maintained in continuous darkness. From test day 32 until test completion, the vessels were illuminated to a light intensity of 137 to 377 lux using fluorescent tubes. A photoperiod of 16 hours was employed with a 30 minute (dawn/dusk) transition period.

Preparation of test solution: A 1 g glyphosate acid/L stock solution was prepared directly prior to test initiation and as required during the exposure period, by dissolving approximately 11.737 g of glyphosate acid in 10 L of dilution water. The stock solution was further diluted (dilution water) by the test item delivery system to achieve the required concentrations in each of the exposure vessels. For the control group, dilution water only without test item was used.

Test units: The test vessels measured 39.0 cm x 19.2 cm, with an approximate water depth of 14.6 cm maintained at a constant volume of 10 L. Two replicates (A and B) were maintained for all treatments and the control.

Test initiation: Prior to fertilization, freshly collected rainbow trout milt and eggs were acclimatized in their respective delivery containers to the approximate test temperature of 12 ± 2°C, using a water bath and then mixed carefully together. The 'apparently' fertilized eggs were impartially distributed to egg incubation cups in groups of five, until each cup contained 50 eggs. The incubation cups were suspended in the respective exposure vessel with two cups per replicate vessel, resulting in 100 eggs per replicate. The test was initiated once all vessels contained eggs within 3.5 hours of receipt of the gametes and within two hours of fertilisation.

Hatching success was determined on days 22 to 26 based on the number of viable eggs. Any eggs exhibiting embryonic development, whether dead or alive, at the time of assessment, were considered fertile for purposes of determining percent viability. All non-viable eggs were counted and discarded at day 26. The percent viability was calculated based on the actual number of fertilized embryos on day 26. Hatching success was calculated based on the actual number of viable embryos.

Egg exposure: Dead and alive eggs were counted daily. All eggs observed to be clear were considered to be alive, all eggs observed to be opaque and milky were considered to be dead. All eggs observed to be dead were removed and preserved in Stockard's solution for clearing and determination of embryonic development. Fry which hatched prior to the determination of viability were collected in an auxiliary egg cup.

Post hatch exposure: At completion of hatch on day 26, twenty organisms per replicate i.e. 40 organisms per treatment level and control were transferred directly from the first egg cup (i.e., A1 and B1) to the surrounding test media in the test vessels and the egg cups were removed.
For replicate A of the control and the 0.095 mg glyphosate acid/L treatment, 20 fry in the auxiliary egg cup containing the early hatched fry were randomly selected. For replicate A of the 10 mg a.s./L treatment, only eight viable eggs hatched of the 20 randomly selected eggs and therefore only eight hatched fry were released into the test vessel.
All remaining alive and dead eggs were preserved in Stockard's solution. The remaining fry were recorded and then discarded. After evaluation of the developmental status of the cleared eggs, the viability of all eggs was calculated.
During the post-hatch exposure period, developing fry in all vessels were observed daily; recording behaviour and appearance. Dead fry were removed during these observations. Survival was estimated daily throughout the post-hatch period. At 60-days post-hatch exposure (experimental completion), the percentage fish survival was calculated.
Fry feeding: At the beginning of fry swim-up, the fry were fed live brine shrimp nauplii (*Artemia salina*), harvested from hydrated cysts (24 to 36 hours post-hydration) three times per day. Fish were not fed during the 24 hours prior to study termination.
Length and weight: At day 60 post-hatch all of the surviving fish in each replicate vessel were euthanized with MS-222 (tricain methane-sulfonate), measured and weighed individually to determine fish total lengths and wet weights, respectively for each treatment.

Observations
The dissolved oxygen (DO) concentrations, pH and temperature were measured and recorded in each test vessel at experimental start and weekly thereafter until test termination (day 85). On test day 75, the DO levels decreased to between 6.31 to 7.50 mg O_2/L, so aeration was provided to each test vessel until test completion.
Temperature was continuously monitored in one replicate (replicate A of the control) throughout the study. Total hardness, alkalinity and specific conductivity were monitored at experimental start and on test days 5, 11, 19, 25, 32, 39, 46, 53, 61, 67, 74 and 81 in one replicate of the highest treatment level and the control during the exposure.

Analytical procedures
Prior to the start of the exposure phase, i.e., day -2, samples from one replicate of the treatment level solutions and control solutions were collected and analysed for the active ingredient. Results of the pre-test analyses were used to assess correct dosage of the system before test initiation.
During the in-life phase, water samples of approximately 10 mL were removed from both replicates of each treatment level and control on test days 0, 6, 13, 20, 27, 33, 41, 48, 55, 62, 70, 76 and 85 and the content of glyphosate acid was determined. Samples of the stock solutions were also analysed at each sampling interval.

Statistical calculations
The data for percent normal fry at hatch, hatching success, survival at test termination and growth (total length, wet and dry weight) were first checked for normality using Shapiro-Wilks' Test (Weber *et al.*, 1989) and for homogeneity of variance using Bartlett's Test (Bartlett, 1937).
The data set for hatching success and survival at test termination were arc-sine (square root) transformed prior to determination of the NOEC and the LOEC by using one-way ANOVA and the parametric post-hoc Dunnett’s Test (Dunnett, 1955, 1964). The data sets for growth passed the tests for homogeneity and normality, and Dunnett’s Test was used to determine the NOEC and the LOEC.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

Analytical data: The mean measured concentrations (calculated as geometric means) of 0.305, 0.977, 3.125 and 10.0 mg a.s./L ranged between 85.7 and 96.3% of the nominal test concentrations, with the exception of the lowest test concentration (0.095 mg a.s./L), where a mean recovery of 66.9% of the nominal concentration was calculated. Based on these results, the mean measured concentrations (calculated as geometric means) of 0.064, 0.261, 0.846, 2.804 and 9.63 mg a.s./L were used for the evaluation of the biological data.

Table B.9.2.2.1-1: Analytical results

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]	% of nominal
Control	-	-
0.095	0.064	66.9
0.305	0.261	85.7
0.97	0.846	86.6
3.125	2.804	89.7
10.0	9.63	96.3

The water quality parameters measured were not affected by test item concentrations. The results of the water quality measurements carried out during this study established that conditions maintained throughout the 85-day exposure were satisfactory for the promotion of normal rainbow trout embryo hatchability, fry survival and growth.

The effects of glyphosate acid on embryo viability, hatching success, number of normal fry at hatch, survival at test termination and growth (total length, wet and dry weight) are provided in the table below.

Table B.9.2.2.1-2: Egg viability, hatching success and normal fry at completion of hatch (test day 26) and survival, total length, wet weight and dry weight of rainbow trout (*Oncorhynchus mykiss*) at test termination of the 85-day exposure to glyphosate acid

Mean measured concentration (mg a.s./L)	Egg viability [%][A]	Hatching success [%][A]	Normal fry at hatch [%]	60 days post-hatch			
				Survival [%]	Total length [mm]	Wet weight [mg]	Dry weight [mg]
Control	35±3.3	92±6.9	97±0.56	85±7.1	46.38±0.41	942.6±34.9	195.1±14.3
0.064	43±4.9	84±20.2	96±5.2	95[B]±7.1	45.33±0.83	899.6±10.7	188.7±5.9
0.261	40±4.0	99±1.7	100±0.0	95±0.0	46.75±0.65	932.2±60.5	190.7±7.5
0.846	38±9.9	95±1.5	100±0.0	93±10.6	46.37±1.7	908.6±84.3	189.1±23.0
2.804	41±2.1	91±5.5	99±2.0	95±7.1	46.19±0.33	889.7±23.7	188.4±10.7
9.63	27±9.2	80±28.3	98±2.1	100±0.0	46.38±1.7	947.3±135	203.0±36.5

[A] Based on total number of viable eggs

[B] On test day 59, one fish of replicate A was inadvertently injured during the cleaning process of the test vessel. One day later this fish had died. Since this mortality was not test item related, the fish was therefore excluded from further statistical evaluation.

The NOEC and LOEC values for survival and growth of rainbow trout (*Oncorhynchus mykiss*) after 85-day exposure to glyphosate acid are based on geometric mean measured concentrations.

Table B.9.2.2.1-3: Endpoints

Endpoint	Glyphosate acid [mg a.s./L]	
	NOEC	LOEC
Percent normal fry at hatch	9.63	>9.63
Hatching success	9.63	>9.63
Survival at test termination	9.63	>9.63
Total length	9.63	>9.63
Wet weight	9.63	>9.63
Dry weight	9.63	>9.63

All validity criteria according to OECD 210 were fulfilled, as dissolved oxygen concentration was between 60% and 100% of air saturation, water temperature was within the range specified for the test species and constant exposure conditions have been maintained (i.e. within ±20% of nominal concentration were recovered, except for the lowest concentration which does not affect the results of the study), and overall survival of fertilised eggs in the controls was greater than or equal to the limits defined in Annexes 3 and 6 of OECD 210.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a 85-day (60 days post-hatch) chronic study with rainbow trout (*Oncorhynchus mykiss*) exposed to glyphosate acid, the NOEC and LOEC values for percent normal fry at hatch, hatching success, fry survival, length and weight were ≥ 9.63 and > 9.63 mg a.s./L, respectively, based on geometric mean measured concentrations.

The study is considered valid and the NOEC for rainbow trout exposed to glyphosate acid was ≥ 9.63 mg a.s./L (nominal) and is considered to be appropriate for use in ecotoxicological risk assessment.

Assessment and conclusion by RMS:
The validity criteria of the OECD 210 (2013) are met.

- hatching success and post hatch success both > 75% (as required for rainbow trout);
- dissolved oxygen concentration >60% of the air saturation value throughout the test;
- According to OECD 210 (2013), the water temperature should not differ by more than + 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for this species (i.e. 10±1.5°C for rainbow trout). In this study the single point temperature measured in the test solutions ranged between 11.3 and 13.9ºC, whereas the continuously measured temperature ranged from 9.4 to 13.1°C throughout the exposure. This slightly exceeds the new recommended range (of 2013 version) but RMS does not consider this deviation would affect the outcome of the study.
- Analytical verifications were made.

This study is valid.

Concentrations have been satisfactorily maintained at all concentrations (i.e. within ±20% of nominal concentration were recovered), except for the lowest concentration. According to OECD 210, when

the measured concentrations do not remain within 80-120% of the nominal concentration, the effect concentrations should be determined and expressed relative to the arithmetic mean concentration for flow-through tests. Geometric mean is then not the most in accordance with the guideline but this has no consequence on the study.

Only 2 replicates per test concentration were used instead of 4 but the number of fish for each concentration was as recommended in OECD 210 (2013).
The results have been reported and statistically analysed per replicates for each tested group.
The Annex 5 of OECD 210 (2013) indicated that : "An individual lab should demonstrate its ability to meet this power requirement either by conducting its own power analysis or by demonstrating that the Coefficient of Variation (CV) for each response does not exceed the 90th percentile of CVs used in developing the TG." The applicant did not provide this demonstration.

RMS notes variability in the egg viability, hatching success and wet and dry weight at the highest concentration of 9.63 mg glyphosate acid/L. The variability observed for dry weight and wet weight in the control is relatively high. The survival was found to be lower in the control than in the tested concentrations. There is no statistically significant difference noted. As only 2 replicates were used, there is uncertainty on the variability. RMS considers the study valid but the robustness of the endpoint is questionable.
No EC10 was calculated. Based on the data available and considering the variability measured at the highest concentration, RMS consider that the estimated EC10 would also be questionable. It was then not calculated by RMS and is not deemed necessary to request.
Overall, RMS considered that the uncertainty is too high to set a robust endpoint. The study is therefore considered valid but not reliable for risk assessment. The reliability of the results is questionable.

Data point:	CA 8.2.2.1/002
Report author	[redacted]
Report year	2000
Report title	Chronic Toxicity of Glifosate Técnico [redacted] to Zebrafish larvae (*Brachydanio rerio*)
Report No	[redacted]-D62.16/99
Document No	
Guidelines followed in study	IBAMA 1990: Manual de testes para avaliacao da ecotoxicidade de agentes quimicos
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations compared from the current OECD 212 guideline (1998):* *Major:* • *The study was not conducted according to the OECD 212 test guideline.* • *Free swimming fish larvae were exposed for 168 h without feeding, therefore the influence of the lack of feeding on the achieved results during the study cannot be excluded.* • *Larvae were added to the test vessels and not fresh eggs 'as soon as possible after fertilisation (early gastrula stage) to 5 days post-hatch (8-10 days) within 30 mins to 8 hours of fertilisation as stated in the test guideline.* • *Active ingredient concentrations were determined in the stock solutions only.* • *Survival of fertilised eggs and differences of water temperature between test chambers or successive days is not reported.* • *Holding stock tank was maintained at 28 ºC. Temperature of test media at fish addition was 24.1ºC. The temperature difference between the holding tank and the test tank, exceeds the variability in temp range permitted for this study type ± 1ºC (25±1ºC stated in Annex 3 of OECD 212).* • *For the batch of eggs received from which the larvae used in the test, were sourced, it is not possible to validate the quality of the eggs used in the test as there is no information on the hatching success reported.* • *Validity criteria based on hatching success and post hatching survival are not reported.*
Previous evaluation	Accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	valid

Summary

A fish short term toxicity test with glyphosate acid with larvae of *Danio rerio* (formerly named *Brachydanio rerio*) was performed under semi-static conditions with test medium each 48 hours. Three replicates with 30 fish per concentration were exposed for 168 hours to seven concentrations of

glyphosate acid, ranging from 0.32 to 32 mg a.s./L. A control treatment containing reconstituted water and a toxic reference using potassium dichromate was maintained concurrently.
Observations for mortality and sub lethal responses were made every 24 hours. Dissolved oxygen, pH and temperature were measured and recorded daily. Glyphosate acid concentrations were measured by liquid chromatography in the stock solutions. Mean measured concentrations were at least 80% of nominal concentrations. Glyphosate acid was not detected in the control group.
A significant increase of mortality was observed at a concentration of 5.6, 10 and 32 mg a.s./L, behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L.

The No-Observed-Effect Concentration (NOEC) for zebra fish (*Danio rerio*) exposed to glyphosate acid was determined to be 3.2 mg a.s./L by the author, who considered that this study is not relevant for use in EU level ecotoxicological risk assessment.
RMS considered the NOEC of 1.0 mg/L relevant for the risk assessment (see commenting boxes)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg acid equivalent

2. Vehicle of test material/media and positive control: Vehicle: Tap water
Positive control: Potassium dichromate ($K_2Cr_2O_7$)

3. Test organism:

Species:	Zebra fish (*Danio rerio*) larvae
Age:	Larvae, approx. 48 hours old
Size:	Not stated
Loading:	1 L for 10 larvae (bodyweight not specified)
Source:	Eggs: in-house. Matrix fish: [redacted]
Diet/Food:	Fish were not fed during acclimation or during the 168 h exposure period.
Acclimation period:	48 hours prior to testing during embryo incubation and hatching

4. Environmental conditions:

Temperature:	23.8 - 24.3 °C
Photoperiod:	16 hours light / 8 hours dark
Dissolved oxygen:	60-100%
Conductivity of test medium:	168 µS/cm
Hardness of test medium:	44.1 mg/L $CaCO_3$

5. Dates of experimental work: 03rd November to 19th November 1999

B. STUDY DESIGN

Experimental treatments
The fish early life-stage toxicity test was performed under semi-static exposure conditions renewing the test solution every 48 hours. Following a range finding test, the freshly hatched fry (48 h post hatch) of *Danio rerio* were exposed to glyphosate acid at test concentrations of 0.32, 0.56, 1.0, 3.2, 5.6, 10 and 32 mg a.s./L for 168 hours. A control consisting of reconstituted water and five toxic reference concentrations (32, 56, 100, 140 and 180 mg $K_2Cr_2O_7$/L) were maintained concurrently.

Observations
Observations for mortality and sublethal responses were made every 24 hours. Dead individuals were removed at each observation. Temperature, dissolved oxygen, pH and conductivity were measured daily. The active ingredient analysis of stock solutions was performed by liquid chromatography.

Statistical calculations
LC_{50} and its confidence limits were determined using trimmed Spearman-Karber method. Fisher's Exact test was used for determination of significant differences in survival between control and exposure. The NOEC and LOEC were determined by Fisher's Exact test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical results: The active ingredient concentration in each stock solution was at least 80% of the nominal concentration. Ecotoxicological relevant endpoints were therefore evaluated using nominal concentrations of the test item.

B. OBSERVATIONS

A significant increase of mortality after exposure to glyphosate acid was observed at concentrations of 5.6, 10 and 32 mg a.s./L. Behavioural responses such as lethargy was observed at 3.2, 5.6, 10 and 32 mg a.s./L. The results of the test are depicted in the following table.

Table B.9.2.2.1-4: Lethal effects of glyphosate acid to zebra fish

Glyphosate acid [mg a.s./L]	**C**	**0.32**	**0.56**	**1.0**	**3.2**	**5.6**	**10**	**32**
Mortality (168 h) [%]	0	0	0	0	10	16.7*	26.7*	56.7*

C = Control
* Added by RMS, significant difference from the control reported in the study report (Fisher test, α=0.05)

For the reference compound potassium dichromate ($K_2Cr_2O_7$) a 168 hour LC_{50} value of 124.66 mg/L (95% C.I. 112.08 – 138.67 mg/L) was determined.

With regard to the validity criteria of the OECD guideline 212 (1998), survival of fertilised eggs and differences of water temperature between test chambers or successive days is not reported. Additionally no information on timing of fertilization is provided. Mortality in control group did not exceed 10%, dissolved oxygen concentration was between 60 and 100% of air saturation. Analysis of test item treatments was performed only for the stock solutions.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The NOEC and the LOEC for zebra fish (*Danio rerio*) exposed to glyphosate acid were determined to be 3.2 mg a.s./L and 5.6 mg a.s./L, respectively, based on nominal concentrations. The LC_{50} after 168 hours was determined to be 24.7 mg a.s./L (nominal).

This study type is based on OECD 212 which is not part of current data requirements and therefore receives a category 5 for studies in AIR 5 dossiers and typically a summary would not be presented. However, for completeness purposes and since the chronic aquatic endpoint in the RAR 2015 was based on this study, it is presented here.

Despite the study having been conducted according to GLP, there are several validity criteria according to the current OECD test guideline 212, that were not fulfilled, with multiple major and minor deviations to the test guideline identified in the summary above, that would make the study unreliable for use in risk assessment.

To further support this evaluation, a further reliability assessment has been conducted using the criteria applied to public domain literature according to EFSA [EFSA Journal 2011;9(2):2092] and is presented in the table below.

Additionally - to ensure an appropriate evaluation of the studies validity and relevance for use in EU level risk assessment, the opinion of an independent Expert is provided in CA 8.2.2.1/003.

Conclusions of the Expert are that this study would not hold up to scientific scrutiny and would not be accepted for a scientific publication.

Based on the reliability assessment and on the opinion of the independent Expert, the study is not therefore considered relevant for use in EU level ecotoxicological risk assessment. Therefore, the study will not be used in ecotoxicological risk assessment for the EU renewal of glyphosate.

ECOTOXICOLOGY: Reliability criteria for the detailed assessment of full-text documents

Data requirements (indicated by the corresponding EU data point)	Criteria for "Reliable" articles	Criteria met? Yes / No / Uncertain
General criteria for reliability considered for all data requirements indicated by the corresponding EU data points as specified in EC Regulation (EU) No 283/2013	1. For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines met.	**Yes** – the study was GLP, but validity criteria of the OECD 212 test guideline were **not** stated / met.
	2. Not previous exposure to other chemicals is documented (where relevant).	**No** – no information in the report to confirm the source / quality of the fish.
	3. For aquatic studies, the test substance is dissolved in water or where a carrier is required, it is appropriate (non-toxic) and a carrier control / positive control is considered in the test design.	**Yes.**
	4. Glyphosate or Its metabolites (AMPA and HMPA), is sufficiently documented , and reported (i.e. purity, source, content, storage conditions)	**Yes**
	5. For tests including vertebrates, compliance of the batches used in toxicity studies compared to the technical specification	**Uncertain** – no information stated in report.

	6. Species used in the experimental clearly reported, including source, experimental conditions (where relevant): strain, adequate age/life stage, body weight, acclimatization, temperature, pH, oxygen (dissolved oxygen for aquatic tests) content, housing, light conditions, humidity (terrestrial species) incubation conditions, feeding.	**No** - Source of fish not stated. Fertilisation and hatching success of egg batches used in test **not** reported. **No** fish body weights reported therefore fish loading rates could not be determined (g fish/L).
	7. The validity criteria from relevant test guidelines can be extrapolated across different species but not necessarily across different test designs. If different, then the nature of the difference and impact should ideally be discussed.	**No** - Validity criteria were not stated. See summary above.
	8. Only glyphosate or Its metabolites is the test substance (excluding mixture), and information on application of the test substance is described.	**Yes**
	9. The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite)	**Uncertain** – Starvation and temperature issues may have also contributed to the observed effects.
	10. Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc.), dilution/mixture of test item (solvent, vehicle) where relevant.	**No** – Definitive test media preparation cannot be confirmed from report - **no** prep details reported. Renewal frequency in the definitive test **cannot** be confirmed. Exposure **cannot** be confirmed in the test system, as there was **no** chemical analysis of test media during the test
	11. Analytical verifications performed in test media (concentration)/ collected samples, stability of the test substance in test medium should be documented	**No** – Test media was not analysed during the test. Report indicates that stock solutions were stable during the test – but this cannot be confirmed from the report
	12. An endpoint can be derived. Findings do deliver a regulatory endpoint, and/or is useful as supporting information	**Uncertain** – as the validity of the test against a relevant guideline set of criteria cannot be confirmed. The test guideline requires freshly fertilized embryos to be

		exposed and not fish larvae – as was the case.
	13. The test has been tested in several dose levels (at least 3) including a positive/negative control where relevant	**Yes**
	14. Suitable exposure throughout the whole exposure period was demonstrated and reported	**No** – there was no analysis of test media during the test
	15. A clearly concentration response relationship is reported – in studies where the dose response test design is employed.	Uncertain – cannot be confirmed as exposure concentrations were not reported
	16. There is included a sufficient number of animals per group to facilitate statistical analysis: mortality in control groups reported, observations/findings in positive/negative control clearly reported (where relevant).	**Yes**
	17. Assessment of the statistical power of the assay is possible with reported data.	**No**
	18. If statistical methodology was applied for findings reported, then the data analysis applied is clearly reported (e.g., checking the plots and confidence intervals)	**Yes**
	19. Description of the observations (including time-points), examinations, and analyses performed, with (where relevant) dissections being well documented.	**No** – detailed timepoint observations of fish and appearance of the test media were not reported.
	20. For terrestrial ecotox studies in the lab or the field, the substrates used should be adequately described e.g. nature of substrate i.e. species of leaf or soil type.	-
	20.1. Field locations relevant/comparable to European conditions. Soils not completely matching the OECD criteria but from Europe or to some extent representative for the European Agriculture. 20.2. Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5%), bulk density, water retention, microbial biomass (~1% of organic carbon)	-
	20.3. Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass	-
	20.4. For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year	-
	20.5. For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max 3 months at 4 +/- 2°C).	-
	20.6. Data on precipitation is recorded	-

	21. For lab terrestrial studies, the temperature was appropriate to the species being tested and generally should fall within the range between 20-25°C and soil moisture / relative humidity was reported.	-
	22. For bee studies, temperature of the study should be appropriate to species.	-
	23. For lab aquatic studies	
	23.1. The source and / or composition of the media used should be described	**Uncertain**
	23.2. The temperature of the water should be appropriate to the species being tested and generally fall within the 15-25ºC	**No** – see deviations section in summary above
	24. The residue data can be linked to a clearly described GAP Table appropriate in the context of the renewal of approval of Glyphosate (crop, application method, doses, intervals, PHI).	**No**
	25. Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate, and where relevant Its metabolites	**No**
	26. Analytical methods clearly described and adequate Statement of specificity and sensitivity of the analytical methods is included.	**No** – There is no analytical method information presented in the report
	27. Assessment of the ECX for the width of the confidence interval around the median value; and the certainty on the level of protection offered by the median ECX.	**Yes** – The presented LC_{50} value is presented with confidence intervals, that exceed the range of concentrations tested in the study. A NOEC is also presented.

<u>Assessment and conclusion by RMS</u>:

The following deficiencies are highlighted by the applicant:

- The applicant argues that *free swimming fish larvae were exposed for 168 h without feeding, therefore the influence of the lack of feeding on the achieved results during the study cannot be excluded*. RMS notes that the guidance OECD 212 recommends not to do so. No feeding is provided in the embryo and sac-fry test, and the test should thus be terminated while the sac-fry are still nourished from the yolk-sac. So this is not seen as a deficiency.

- *Larvae were added to the test vessels and not fresh eggs 'as soon as possible after fertilisation (early gastrula stage) to 5 days post-hatch (8-10 days) within 30 mins to 8 hours of fertilisation as stated in the test guideline*. RMS does not consider this would lead to an overestimation of the effects.

- *Active ingredient concentrations were determined in the stock solutions only*. **RMS usually considers this as a major drawback of the study.** In semi-static tests where the concentration of the test substance is expected to remain within ± 20% of the nominal (i.e.

within the range 80 - 120 %) it is recommended that, as a minimum, the highest and lowest test concentrations be analysed when freshly prepared and immediately prior to renewal on at least three occasions spaced evenly over the test. See later for further analysis of this point.

- *Survival of fertilised eggs and differences of water temperature between test chambers or successive days is not reported.* As mentioned above by RMS, the fact that larvae (and not eggs) were used is not likely to lead to an overestimation of the effect.

- *Holding stock tank was maintained at 28 ºC. Temperature of test media at fish addition was 24.1ºC. The temperature difference between the holding tank and the test tank, exceeds the variability in temp range permitted for this study type ± 1ºC (25±1ºC stated in Annex 3 of OECD 212).* OECD 212 recommends that the temperature in the holding tanks should be maintained at 25 ± 2 °C. So this temperature only slightly exceeded the recommended temperature and this does not seem to have affected the fish (no fish was affected in control group).

- *For the batch of eggs received from which the larvae used in the test, were sourced, it is not possible to validate the quality of the eggs used in the test as there is no information on the hatching success reported.* As mentioned above, the fact that larvae (and not eggs) were used is not likely to lead to an overestimation of the effect.

- *Validity criteria based on hatching success and post hatching survival are not reported. As* mentioned above, the fact that larvae (and not eggs) were used is not likely to lead to an overestimation of the effect. Post hatching survival in the control was 100%.

Further drawbacks are highlighted in the Table above "*Reliability criteria for the detailed assessment of full-text documents*":

- *Not previous exposure to other chemicals is documented (where relevant).* RMS acknowledges that absence of previous exposure cannot be confirmed based on the data available in the report.

- *No fish body weights reported therefore fish loading rates could not be determined (g fish/L).* Dissolved oxygen concentration was not particularly high but globally remained within the requested range of 60-100 % ASV (occasionally it dropped slightly below the recommended range, RMS considers the impact negligible as this deviation occurred also in low concentrations and control without effect on mortality).

- *Exposure cannot be confirmed in the test system, as there was no chemical analysis of test media during the test.* As mentioned above, RMS considers this absence of verification as a major drawback of the study.

RMS considers the absence of analytical verifications as the only major drawback of the study. However the test item appears very stable in the stock solutions* (based on daily measurements during 6 days in the stock solutions, with 97.02 and 100.78% of nominals). Renewal intervals for the test solution were made every 48 h.

* The quality of the chemical analysis cannot be assessed here but stability of the test item can be assumed for this substance in this study design.

Besides a clear dose-effect relationship was observed on 5 (out of the 7) concentrations tested. This seems to indicate correct dosing of the test item. Then, RMS considers that the exposure was adequate and that the results of this study can be used for the risk assessment.

In the guideline OECD 212 it is recommended that the duration of the test should be 8-10 days (Annex 3). In the present study duration was of 7 days. However this study was not meant to follow the recommendations of the OECD 212. Moreover in view of the effects reported with the clear dose response on mortality and the time course of effects (see table below) RMS considered that increase of study duration may have influence the results. Underestimation of effects could not be excluded her and should be considered when setting the NOEC.

Significant increase of mortality reported as statistically significant in the study report (Fisher test) was observed at a concentration of 5.6, 10 and 32 mg a.s./L.
The LC50 after 168 hours was determined to be 24.71 mg a.s./L.

The No-Observed-Effect Concentration (NOEC) for zebra fish (*Danio rerio*) exposed to glyphosate acid was determined by the author to be 3.2 mg a.s./L (nominal). Nevertheless, as previously agreed in RAR 2015, the mortality effect in the study with *Danio rerio* followed a dose response relationship and in the treatment level at 3.2 mg/L a mortality of 10% was observed. Lethargy was also observed at this concentration.
The details of information on mortality and lethargy throughout the time for all concentrations are reported in the table below.

	Glyphosate acid [mg a.s./L]							
	Control	**0.32**	**0.56**	**1.0**	**3.2**	**5.6**	**10**	**32**
	Cumulative number of dead* (other signs : N normal, L lethargy)							
24h	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)
48h	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)
72h	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	6 (N)
96h	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	0 (N)	1 (L)	7 (L)
120h	0 (N)	0 (N)	0 (N)	0 (N)	2 (L)	2 (L)	3 (L)	12 (L)
144h	0 (N)	0 (N)	0 (N)	0 (N)	2 (L)	2 (L)	3 (L)	12 (L)
168 h	0 (N)	0 (N)	0 (N)	0 (N)	3 (L)	5 (L)**	8 (L)**	17 (L)**

* On a total of 30
** Statistical difference reported

RMS considered that lethargy is a severe effect of biological significance and should therefore be considered. It is acknowledge that the effect was not well described, in particular the number of affected fish was not mentioned.
Lethargy occurred at 3.2 mg/L and above at the same time as mortality. There is a clear dose-response on mortality. Moreover, mortality was seen to increase with time at concentrations greater and equal to 3.2 mg/L and lethargy appeared at the same time in these tested concentrations.
Overall, RMS considered appropriate to set the NOEC at 1 mg/L considering that 10% mortality is observed after the 7-day exposure period with clear increase with time.
As lethargy occurred at same time as mortality which exhibit a clear dose-response and increase in time, RMS considered that the 10% effects on mortality should be considered as biologically relevant.

RMS still considers the NOEC of 1.0 mg/L relevant for the risk assessment.
No LC10 was calculated.
A data gap is set to provide a statistical re-analysis (NOEC, LC10/20). Moreover the extent of lethargy should be provided.

Data point:	CA 8.2.2.1/003
Report author	[redacted]
Report year	2020
Report title	External expert opinion to the study No. RF-D62.16/99
Report No	-
Document No	-
Guidelines followed in study	None
Deviations from current test guideline	Not applicable
Previous evaluation	No
GLP/Officially recognised testing facilities	No, not applicable

2. General evaluation

The study by [redacted] (2000) (CA 8.2.2.1/002) was evaluated by an independent fish expert not associated with industry. The expert was not provided with the name of test substance, study director, performing laboratory nor the data owner or sponsor of the study.

The following observations and statements were made:

Overall, the study has been based on OECD TG 212. However, there are several shortcomings in the study or in the report:

(1) A major deviation is that the renewal intervals for the test solution has been extended from 24 h to 48 h. Basically, this can be done; however, in such a case, precise chemical-analytical data documenting the stability of the test solutions must be provided. A general reference such as "[redacted] 1998 (P. 6 of report) is certainly not sufficient and is inadequate. Given that the report lacks any chemical-analytical data, the extension of the renewal time of the test solutions is a serious deviation from OECD TG 212. Since the reviewer was not provided with the name of the test substance, he could not check whether such an extension can be accepted as an exception. In any case, the extension of the renewal time of the test solutions should have explicitly been reported as a deviation from the guideline.

(2) Another critical deviation from OECD TG 212 is the fact that the information about the age of the embryos upon initiation of chemical exposure is confusing, if not lacking. Both OECD TG 212 and the more recent OECD TG 236 clearly require an exposure start as early as possible, if not within the first 1 h after fertilization. The report does not provide any information about the exact timing of the fertilization process and the time of egg collection.

(3) Further deviations from OECD TG 212 are a pH of ~ 7.4 (recommendation OECD TG 212: 7.8) and a temperature of ~ 24 °C (recommendation OECD TG 212: 28 °C). The consequences of these deviations cannot be assessed, as long as the name of test substance is not disclosed. Given the rather wide limits of tolerance of the zebrafish embryo, both pH and temperature deviations may have had an impact on the outcome of the test (chemical speciation, metabolism), however not necessarily.

(4) The terminology for the general description of the assay is scientifically not correct: OECD TG 212 does not measure chronic toxicity, nor does it use larvae.

(5) The origin of the fish is very poorly defined: no information about the strain of zebrafish used, no information about the age of the parental fish.

(6) Likewise, the report lacks data on fertilization rate, which is an important parameter to assess the quality of the egg batch used for the experiment (cf. information required for, e.g., OECD TG 236 [fish embryo test]). Maybe, in 1999, this was acceptable; today it would be not

(7) The report completely fails to provide details on behavioural observation; the term "lethargy" is definitely not satisfying and could have been specified much more precisely.

Additional specific comments:

(1) Although OECD TG 212 also mentions zebrafish as *Brachydanio rerio*, the title of this species has been changed to *Danio rerio*.
(2) The term "larvae" should be avoided for the early developmental stages used in this study. The official title of OECD TG 212 also reads "Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages". Seven days old individuals of zebrafish are scientifically correctly termed "eleutheroembryos", since they still live on the remnants of the yolk, but have not yet completely initiated external food uptake
(3) The term "chronic toxicity" should be avoided, since OECD TG 212 does not use this term for the test itself. OECD TG 212 explicitly states that "Guideline does not replace Guideline 210 but it would provide useful information in that it could (a) form a bridge between lethal and sublethal tests, (b) be used as a screening test for either a Full Early Life Stage test (Guideline 210) or for chronic toxicity
(4) Composition of reconstituted water is missing.
(5) Lack of information on the strain, age of the fish used for egg production.
(6) Lack of information on the parental fish: the water used for the maintenance, maintenance conditions, composition of breeding groups (Loading).
(7) The quality of the chemical analysis cannot be assessed, since reference to an internal SOP is not sufficient as long as the SOP is not provided
(8) Oxygen saturation occasionally drops below 60 % (e.g. 4.4 mg/L in some replicates of 0.56 mg/L test solution, which is equivalent to 53 % [saturation: 8.3 mg/L at 23.5 °C]). The minimal acceptable oxygen saturation for OECD TG 212 is 60 %. Since such low oxygen saturation were measured repeatedly (Table p. 30 of report), this parameter is somewhat borderline

Summarized Deviations from the test guideline:

As per deviations compared from the current OECD 212 guideline (1998):
Major:

- The renewal intervals for the test solution has been extended from 24 h to 48 h.
- The information about the age of the embryos upon initiation of chemical exposure was confusing, if not lacking.
- pH of ~ 7.4 (recommendation OECD TG 212: 7.8) and a temperature of ~ 24 °C (recommendation OECD TG 212: 28 °C).

Minor:

- Lack of data on fertilization rate.
- No details on behavioural observation provided

Given these major problems and the relatively long list of specific comments listed below, **this report would not be acceptable as a *scientific publication*.**

Assessment and conclusion by applicant:
According to expert opinion, study RF-D62.16/99 would not be accepted for scientific publication due to various deficiencies and should also not be considered a chronic study in the assessment of effects of glyphosate on fish.
As a publication, this study would not be considered reliable and would not be considered for risk assessment.

Assessment and conclusion by RMS:
The deficiencies highlighted in this opinion were discussed above (previous commenting box by RMS).
RMS acknowledges that the age of the embryos upon initiation of chemical exposure was not reported. Then, this study may not cover the earliest development stage of the fish. However the effect on mortality that were observed cannot be ignored whatever the development stage of the fish tested in this study.

Data point:	CA 8.2.2.1/004
Report author	[redacted]
Report year	2011
Report title	AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*)
Report No	139A-39A
Document No	-
Guidelines followed in study	OECD Guideline 210 (1992) OPPTS 850.1400 ASTM E 1241-05
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current OECD 210 guideline (1992): none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Summary

The effects of AMPA (Aminomethyl-phosphonic acid) on the time of hatch, hatching success, survival and growth of fathead minnow (*Pimephales promelas*), was evaluated in a fish early life-stage toxicity test performed under flow-through exposure conditions, using a continuous flow test item delivery system. The appropriate test medium was supplied to four replicates at each of five concentrations and a negative control (dilution water only) group. The fertilized eggs were exposed to AMPA at nominal test concentrations of 0.75, 1.5, 3.0, 6.0 and 12 mg/L for a 5 day hatching period followed by a 28 day post hatch growth period.
AMPA concentrations in test media were measured on day 0, 7, 14, 21, 28 and 33. Mean measured concentrations ranged from 82.5 to 117% of nominal concentrations. AMPA was not detected in the control group.
No significant differences in the time to hatch, hatching success, survival at test termination and growth (total length, wet and dry weight) were observed, when compared to the control. All validity criteria according to the current guideline OECD 210 were fulfilled.
In an fish early life stage test (OECD 210), performed using fathead minnows (*Pimephales promelas*) the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC) for fathead minnow (*Pimephales promelas*) exposed to AMPA were determined to be 12.0 and > 12.0 mg/L, respectively, based on mean measured concentrations. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7%

2. Vehicle of test material/media: moderately hard well water

3. Test organism:

Species:	Fathead minnow (*Pimephales promelas*) embryos <24 hours old
Age of eggs:	<24 hours old
Number of animals/dose level:	20 organisms per replicate i.e. 80 organisms per treatment level and control
Supplier:	[redacted]
Mean loading rate (biomass per volume of test solution)	0.05 g fish/L per 24 hours; instantaneous loading at the end of test: 0.32 g fish/L
Diet/Food:	live brine shrimp nauplii (*Artemia* sp.), Brine Shrimp Direct, Ogden, Utah, USA

4. Environmental conditions:

Temperature:	25±1°C
pH:	7.8 to 8.2
Dissolved oxygen:	≥ 89% of saturation (7.3 mg/L)
Conductivity of test medium:	361 - 395 µS/cm
Hardness of test medium:	132 - 140 mg/L $CaCO_3$

Photoperiod: 16 hours with a 30 minute transition period;
Light intensity = 296 lux

5. Dates of experimental work: 13th January to 03rd February 2011

B. STUDY DESIGN

Experimental treatments

The fish early life-stage toxicity test was performed under flow-through exposure conditions, using a constant-flow test item delivery system, supplying the appropriate test medium to the exposure vessels at each of the five concentrations and a negative control (dilution water only) group. The embryos of fathead minnow (*Pimephales promelas*) were exposed to AMPA at test concentrations of 0.73, 1.5, 2.9, 6.0 and 12.0 mg/L for 33 days. The test was conducted in a temperature controlled environmental chamber. The test vessels were 9 L glass aquaria with a constant volume of 7 L of test solution. Embryos were held in incubation cups constructed from glass cylinders 50 mm in diameter with 425 µm nylon screen mesh. Four replicates vessels were maintained for all treatments and the control.
At test initiation, embryos <24 hours old were impartially distributed to incubation cups. After a hatching period of 5 days, larvae were released into test chambers. Newly hatched larvae were fed live brine shrimp nauplii (*Artemia sp*.) harvested from hydrated cysts 2 - 3 times per day.

Observations

During the first day of exposure, embryos were observed twice for mortality and fungal infection. Thereafter, until hatching was complete, observations of embryo mortality and the removal of dead embryos was performed once per day. Once hatching had reached >90% in the control groups on day 5 of the test, the larvae were released into their respective test vessels and the post-hatch period began. During the 28-day post-hatch exposure period, the number of fry mortalities and numbers of individuals exhibiting clinical signs of toxicity or abnormal behaviour was recorded. From these observations, the time to hatch, hatching success, and post-hatch growth and survival were evaluated. On day 28 of the post-hatch exposure period – test termination, the total length for all surviving fish was measured to the nearest 1 mm using a metric ruler and wet and dry weights of all fish was measured to the nearest 0.1 mg using an analytical balance. Fish were euthanized (MS-222) and dried to constant weight in an oven at approximately 60°C for approximately 47 hours to establish fish dry weight data.
Dissolved oxygen, temperature and pH were measured in alternating replicates of each treatment and control group at the beginning of the test, weekly during the test, and at the end of the test. Hardness, alkalinity and specific conductance were measured in alternating replicates of the negative control (dilution water) and the highest concentration treatment group at the beginning of the test, weekly during the test and at the end of the test.

Analytical procedures

Analytical measurements were performed by HPLC analysis using UV detection. Water samples were collected from one test chamber of each treatment and control group four days prior to test initiation to confirm the operation of the diluter. Water samples were collected from alternating replicate test chambers of each treatment and control group on day 0, 7, 14, 21, 28 and 33 (test termination) to determine concentrations of the test substance in the test chambers. All samples were collected at mid-depth in the test chambers, placed in glass vials and analysed immediately.

Statistical calculations

Data were statistically tested using Chi-square and Fisher's Exact test (discrete-variable data; $\alpha = 0.05$) and Dunnett's t-test (one-tailed, normal distributed data; $\alpha = 0.05$).The NOEC and LOEC were determined by visual interpretation of the observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical measurements were performed on samples of representative test concentrations. Recoveries ranged from 82.5 % to 117% relative to nominal concentrations for all test concentrations and ranged from 97 to 100% of nominal for overall mean measured concentrations.

Table B.9.2.2.1-5: Analytical results

Nominal concentration of AMPA [mg/L]	Mean measured concentration of AMPA [mg/L]	% of nominal
Control	Control	-
0.75	0.73	97
1.5	1.5	100
3.0	2.9	97
6.0	6.0	100
12	12	100

The water quality parameters measured were not affected by test item concentrations. The results of the water quality measurements carried out during this study established that conditions maintained throughout the 33-day exposure were satisfactory for the promotion of normal fathead minnow embryo hatchability, fry survival and growth.

B. OBSERVATIONS

The effects of AMPA on embryo viability, hatching success and growth (total length, wet and dry weight) are provided in the table below.

Table B.9.2.2.1-6: Hatching success, larval survival and total length, wet weight and dry weight of fathead minnow (*Pimephales promelas*) at test termination of the 33-day exposure to AMPA.

Mean measured concentration of AMPA [mg/L]	Hatching success [%]	Survival to day 28 post hatch [%]	Growth 28 days post-hatch		
			Mean total length [mm]	Mean wet weight [mg]	Mean dry weight [mg]
Control	99	91	25.2 ±0.57	112.0 ±11.5	24.1 ±1.4
0.73	100	91	25.2 ±0.27	120.7 ±7.4	24.6 ±1.0
1.5	100	93	25.5 ±0.39	119.3 ±14.2	24.9 ±2.1
2.9	100	90	25.7 ±0.62	117.4 ±3.8	23.5 ±0.42
6.0	100	91	25.4 ±0.22	117.4 ±4.2	23.6 ±0.70
12	99	92	26.2 ±0.62	135.2 ±11.0	26.5 ±2.9

The majority of the fish in the control group and in the AMPA treatment groups appeared normal throughout the test. Through Day 7 post-hatch, in the control group and in the AMPA treatment groups, a low frequency of larvae were noted as either weak, lying on the bottom of the test chambers, curled, or having a curled or curved spine/crooked spine. The frequency of curved/curled or curled spine/crooked spine observed in the treatment groups were comparable to historical frequencies observed in control

treatments in early life-stage studies with fathead minnows performed at the test facility and consequently concluded to be not treatment related. Additionally, the frequencies of the occurrence of smaller fish visually observed in the control and treatment groups were comparable and consistent with the individual dry weight measurements.

The 33-day NOEC values are given below based on mean measured concentrations.

Table B.9.2.2.1-7: Endpoints table

Endpoints (33 days)	AMPA [mg/L]
LOEC (hatching success, survival or growth)	>12
NOEC (hatching success, survival or growth)	12

All validity criteria according to OECD 210 were fulfilled, as dissolved oxygen concentration was between 60% and 100% of air saturation, water temperature was within the range specified for the test species and constant exposure conditions have been maintained (i.e. within ± 20% of nominal concentration were recovered), and overall survival of fertilised eggs/embryos in the controls was greater than or equal to the limits defined in Annexes 3 and 6 of OECD 210.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a fish early life stage test (OECD 210) performed using fathead minnow (*Pimephales promelas*) exposed to AMPA, the NOEC and LOEC values for hatching success, fry survival, length and weight were ≥ 12 and >12 mg/L, respectively, based on mean measured concentrations.

The study is considered valid and the NOEC for fathead minnow exposed to AMPA was ≥ 12 mg/L (mean measured concentrations) and is considered to be appropriate for use in ecotoxicological risk assessment.

Assessment and conclusion by RMS:
This study is considered valid.

In accordance with the TG 210 (2013) an ability to meet an appropriate statistical power should be demonstrated with the calculation of the Coefficient of variation (CV) for each response between and within replicates. A statistical power analysis as presented in appendix 5 of the OECD 210 guideline could be useful to confirm the robustness of the NOEC. A data gap should be set.

NOEC for fathead minnow exposed to AMPA = 12 mg/L (mean measured concentrations)
No EC10 was provided.
As no effect was observed, RMS does not consider that EC10 is necessary.

Data point:	CA 8.2.2.1/005
Report author	Rodrigues, L.B. *et al.*
Report year	2019
Report title	Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms

Document No	doi.org/10.1016/j.mrgentox.2019.05.002 E-ISSN: 1873-135X
Guidelines followed in study	OECD 236
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Not reported*
GLP/Officially recognised testing facilities	No
Acceptability/Reliability **(RMS)**:	Yes/Reliable with restrictions

For detailed summary of this article proposed as relevant and reliable by both applicant and RMS please refer to the appendix to Volume 3 CA B.9 on literature data related to ecotoxicologyunder point B.9.2.2.

Data point:	CA 8.2.2.1/006
Report author	Schweizer, M. *et al.*
Report year	2019
Report title	How glyphosate and its associated acidity affect early development in zebrafish (*Danio rerio)*
Document No	DOI 10.7717/peerj.7094 ISSN: 2167-8359
Guidelines followed in study	OECD Guideline 236
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*None*
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability (RMS):	Relevant and Reliable with restrictions

For detailed summary of this article proposed as relevant and reliable by both applicant and RMS please refer to the appendix on literature data of Volume 3 CA under point B.9.2.2.

B.9.2.2.2. Fish full life cycle test

Data point:	CA 8.2.2.2/001
Report author	Anonymous
Report year	1975
Report title	Chronic Toxicity of Glyphosate to the Fathead Minnow (*Pimephales promelas*, Rafinesque)
Report No	BN-75-129
Document No	-
Guidelines followed in study	EPA: Recommended bioassay procedures for fathead minnow (Pimephales promelas, Rafinesque) chronic tests. By the Bioassay Committee, National Water Quality Laboratory, Duluth, USA (1971)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the current EPA guideline OPPTS 850.1500 (1996):* *- none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Supportive

Summary
The effects of glyphosate on fathead Minnow (*Pimephales promelas*) were evaluated in a full life cycle test in flow-through test conditions. The test was performed using mean measured concentrations of 0.7, 2.8, 7.0, 13.0 and 25.7 mg glyphosate/L (mg a.s./L). In addition, a control group was exposed to the dilution water. At test initiation, thirty fathead minnow eggs were incubated in each test aquarium and observed for effects at all developmental steps of the full life cycle. Forty fish were divided into two groups of twenty each, were randomly selected, and distributed to growth chambers in each aquarium. Two growth chambers were used to facilitate handling of fry for 30 and 60 day measurements by a photographic method. Percent survival based on cumulative mortality was also determined at these intervals. After 60 day measurements, the number of fish released to each spawning chamber was impartially reduced to fifteen after combining fish from the growth chambers. When secondary sexual characteristics were well developed (circa day 134), the number of fish in each tank was reduced initially to four males and four females and subsequently (day 179) to two males and four females which were allowed to spawn.
During the full life cycle test, adult fecundity (approx. day 112) and survival (day 30, 60 and day 134) were recorded. The egg hatchability was determined on the first generation eggs 4 days after the test initiation. Total length (day 30, day 60 and day 255), total wet weight (day 254), sex ratio (day 134 and day 254) and gonadal conditions (day 254) were equally determined for each adult fish.
Temperature and dissolved oxygen were measured on a daily basis. The alkalinity, acidity and hardness of the test water were measured on a weekly basis. None of the parameters studied (adult fecundity, parental and juvenile mortality, total length, wet weight, sex ratio and gonadal conditions), were significantly affected by the chronic exposure to the test item.
The study is considered informative only by RMS.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	None
Lot/Batch #:	Not stated
Purity:	87.3%

2. Vehicle of test material/media: dilution water

3. Test organism:

Species:	Fathead minnow (*Pimephales promelas*, Rafinesque)
Age:	Not stated
Size:	Not stated
Loading:	40 fish per aquarium of 41 L test solution (at test initiation)
Source:	In-house stock culture
Diet/Food:	3 - 4 times per day *ad libitum* with brine shrimp nauplii (first 45 days); Twice a day *ad libitum* with frozen brine shrimp (after 45 days)
Acclimation period:	Not stated
Body weight of the animals:	1.5 g

4. Environmental conditions:

Temperature:	25 ± 1°C (chronic test)
Photoperiod:	16 hours light / 8 hours dark
pH:	6.5 – 7.6
Dissolved oxygen:	6.3 – 9.0 mg O_2/L
Conductivity:	not stated
Hardness:	32 - 42 mg $CaCO_3$/L

5. Experimental dates of work: Test start: January 27th 1975

B. STUDY DESIGN

Experimental treatments: A fish chronic toxicity tests (full life cycle) was performed with glyphosate using concentrations 0.7, 2.8, 7.0, 13.0 and 25.7 mg a.s./L (mean measured) in a flow-through test. In addition, a control group was exposed to the dilution water. The test medium in aquaria was exchanged continuously through a flow-through system. A glass flow-splitting chamber was calibrated to deliver an equal flow rate to the growth chambers. There were six duplicate test vessels, containing 41L test solution each. At test initiation, thirty eggs were incubated in each test vessel. Dead eggs were removed and counted each day until hatching was completed (4 days at 25°C). 40 fish (selected from the hatched fish) were randomly distributed to growth chambers in each vessel. Percent survival based on cumulative mortality was determined at these intervals. After 60 day, the number of fish released to each spawning chamber was impartially reduced to fifteen after combining fish from the growth chambers. On day 64, five spawning sites were made. When secondary sexual characteristics were well developed (circa day 134) the number of fish in each tank was reduced initially to four males and four females and subsequently (day 179) to two males and four females. When spawning began (circa day 112), eggs

were daily removed from the underside of spawning tiles and counted. Fifty eggs from each of the first ten spawning were then oscillated in their respective test waters and dead eggs were removed and counted daily, until hatching was completed. Twenty fry from the first two spawns in each tank, in which at least 80% live hatch was observed, were placed in their respective growth chambers and observed for 30 days, after which fry groups were terminated and total lengths determined by the photographic method. Total length, wet weight, sex and gonadal conditions were determined for each adult fish at the termination of the experiment.

Observations: During the full life cycle test, adult fecundity was determined approximately on day 112 and survival was observed on day 30, day 60 and day 134. The egg hatchability was determined on the first generation (F1) eggs 4 days after the test initiation. Total length, wet weight, sex and gonadal conditions were equally determined for each adult fish at termination of the experiment after 254 days. Temperature and dissolve oxygen were measured on a daily basis. The alkalinity, acidity and hardness of the test water were measured on a weekly basis. Chemical analyses were performed on samples of the test solutions (taken weekly) to quantify glyphosate in test solution with colorimetric measurements. Indirect quantification of glyphosate was used by quantifying ortho-phosphate and total phosphorus, and then to correct the quantification of the difference between the two analyses for background (i.e. controls) and results were expressed as mg/L phosphorus calculated as glyphosate.

Statistical calculations: ANOVA, Duncan's Multiple Range Test at $\alpha = 0.05$ as post hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.2.2.2-1: Endpoints

Endpoints	Glyphosate [mg a.s./L]
NOEC (255 days)	25.7

Analytical results: Chemical analyses were performed on samples of the test solutions (taken weekly) to quantify glyphosate in test solution. The mean measured concentrations of the test item in test solutions were 43.75%, 87.50%, 110.11%, 104.0% and 102.80% for the nominal test concentrations of 1.6, 3.2, 6.3, 12.5 and 25 mg a.s./L respectively.

Table B.9.2.2.2-2: Analytical results

Nominal concentration of glyphosate [mg a.s./L]	Mean measured of glyphosate [mg a.s./L]	% of nominal
Control	-	-
1.6	0.7	43.8
3.2	2.8	87.5
6.3	7.0	110.1
12.5	13.0	104.0
25.0	25.7	102.8

B. OBSERVATIONS

Clinical observations: Analyses of variance indicated that continuous exposure of fathead minnows to concentrations of glyphosate as high as 25.7 mg a.s./L had no significant effects on any of the parameters studied during 254 days of continuous exposure. Hatchability of eggs was >94% in all test item treatments. Mortality and total length of fathead minnows after 30 and 60 days of exposure to concentrations of glyphosate in the treatment groups did not differ significantly from control fish. No effect was observed through day 134 on the survival of the 15 original fish placed in each spawning

chamber. At termination, total length and wet weight of the female fathead minnows were similar to controls among fish exposed to all concentrations of glyphosate. The number of spawning, eggs per female and eggs per spawn did not differ significantly between controls and fish exposed to the test item treatments.
Percentage of live fry hatching in test item treatments was similar to that which was observed in the controls. Survival and total length and wet weight of second generation fathead minnows was similar to controls for fish exposed 30 days to concentrations of glyphosate. The number of spawnings, eggs per female and eggs per spawn did not differ significantly between controls and fish exposed to concentrations of glyphosate as high as 25.7 mg/l. One spawn of 33 eggs was recovered from the B replicate of 25.7 mg/l before the accidental death of fish due to a diluter malfunction early in the spawning period. Prior to that time, all fish appeared healthy and had reached sexual maturity.

Table B.9.2.2.2-3: Survival and growth of fathead minnows during chronic exposure to glyphosate (mean values)

Glyphosate (mg a.s./L)		Control	0.7	2.8	7.0	13.0	25.7
Egg hatchability		99.5	97	96	97	99	97
Day 30	Survival [A]	93	81.5	78	89	73	89
	Total length	16	16	16	14.5	16.5	16
Day 60	Survival	93	81.5	76.5	82.5	73	89
	Total length	25.5	25	27.5	26.5	27.5	26
Day 134	Survival [B]	100	93	96.5	96.5	76.5	96.5
Day 254	Total length ♂	59	62	62	63	65	61 [C]
	Total length♀	47	46	48	45	48	42 [C]
	Total weight ♂	2.82	3.3	3.4	3.0	3.2	2.4 [C]
	Total weight ♀	1.02	1.03	1.18	0.91	1.05	0.94 [C]

[A] Survival based on 40 fish per duplicate.
[B] Survival based on 15 fish per duplicate.
[C] Fish accidentally killed on day l68 due to diluter malfunction (duplicate B). Results from duplicate A are reported.

Table B.9.2.2.2-4: Spawning and egg hatchability of fathead minnows continuously exposed to glyphosate (mean values)

Glyphosate (mg a.s./L)	Control	0.7	2.8	7.0	13.0	25.7 [A]
Number females	4	4	4	4	4	~~4~~ RMS: 4 (A)/4 (B)
Spawning/♀	9.5	4.5	10.0	5.5	5.0	~~4.5~~ RMS: 8 (A)/<1 (B)
Eggs spawned/♀	~~340~~ RMS: 340 (A)/960 (B)	207	619	323	298	~~263~~ RMS: 518 (A)/8 (B)
Eggs/spawning	66.5	51.0	62.5	60.0	65.0	~~51.0~~ RMS: 69 (A)/33 (B)
Hatchability	93.5	90.0	87.5	91.5	89.5	~~86.5~~ [B] RMS: 91 (A)/82 (B)
N [C]	7.5	6.0	10.0	9.0	5.5	~~6.0~~ RMS: 9 (A)/3 (B)

[A] All fish killed on day 168 due to diluter malfunction in only one compartment of the aquarium (duplicate B). RMS does not accept the mean values calculated by the applicant and results from both duplicates were reported by RMS.
[B] Eggs from unexposed parents (in the aquarium compartment, in which all fish were killed)
[C] Number of egg groups exposed.

The following table was not reported in the study summary provided by the applicant. So RMS reported it below for completeness.

Table B.9.2.2.2-5: Survival and growth of F1 fathead minnows continuously exposed to glyphosate for 30 days.

Glyphosate (mg a.s./L)	Control	0.7	2.8	7.0	13.0	25.7
Survival (%)[A]	80 (A)/90 (B)	85 (A)/98 (B)	90 (A)/75 (B)	100 (A)/98 (B)	95 (A)/95 (B)	95 (A)/90 [B] (B)
Total length (mm)	20 (A)/19(B)	21 (A)/19 (B)	19 (A)/20 (B)	20 (A)/19 (B)	20 (A)/17 (B)	21 (A)/19 (B)
Standard deviation	(2.6) (A)/(4.0) (B)	(3.3) (A)/(4.1) (B)	(3.4) (A)/(3.5) (B)	(3.1) (A)/(2.5) (B)	(3.3) (A)/(2.8) (B)	(2.9) (A)/(2.7) (B)
Total weight (mg)	64 (A)/66 (B)	91 (A)/60 (B)	59 (A)/73 (B)	73 (A)/63 (B)	76 (A)/50 (B)	74 (A)/59 (B)

[A] mean of two larval groups of 20 per duplicate
[B] Fry from unexposed parents

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a flow through full life cycle study of fathead minnows exposed to glyphosate, none of the parameters studied (adult fecundity, parental and juvenile mortality, total length, wet weight, sex ratio and gonadal conditions), were significantly affected by the chronic exposure to glyphosate. The NOEC was determined to be ≥ 25.7 mg a.s./L (mean measured).

This flow through full life cycle study is considered valid and the NOEC value for fathead minnow exposed to glyphosate was determined to be >25.7 mg a.e./L (mean measured) and can be used in risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was not conducted under GLP (GLP was not compulsory at the time the study was performed).
Indirect quantification of glyphosate was used by quantifying ortho-phosphate and total phosphorus, and then to correct the quantification of the difference between the two analyses for background (i.e. controls) and results were expressed as mg/L phosphorus calculated as glyphosate. The derivatisation efficiency of this analytical method can not be verified by RMS. The full validation report was not available. Thus the analytical part of the study should be considered with caution.
Some parameters show high variability between duplicates (e.g. eggs spawned/♀) and results appears sometimes fluctuant even if no obvious trend for effect is observed. The reliability of the statistics is doubtful and potential effects could have been masked.
Nevertheless RMS agrees that this study does not show any evidence for effect even at the highest concentration (25.7 mg a.e./L).
RMS considers this study as informative only and not reliable enough to set a robust endpoint.
No effect on survival, growth or reproduction of adult fathead minnow or progeny were observed when exposed to concentrations of up to 25.7 mg a.e./L for up to 8 months (255 days).
The study is considered informative only.

B.9.2.2.3. Bioconcentration in fish

Data point:	CA 8.2.2.3/001
Report author	[redacted]
Report year	1989
Report title	Uptake, Depuration and Bioconcentration of ^{14}C Glyphosate to Bluegill Sunfish (*Lepomis macrochirus)* Part I
Report No	[redacted]-9304
Document No	-
Guidelines followed in study	Guideline 72-6
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations according to the current OECD 305 guideline (2012):* *- none.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes

Data point:	CA 8.2.2.3/002
Report author	[redacted]
Report year	1989
Report title	Uptake, Depuration and Bioconcentration of ^{14}C Glyphosate to Bluegill Sunfish (*Lepomis macrochirus)* Part II: Characterization and Quantitation of Glyphosate and Its Metabolites
Report No	[redacted]-9303
Document No	-
Guidelines followed in study	Guideline 72-6
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations according to the OECD guideline 305;* *Minor: fish loading range of 0.1 g – 1.0g/L, actual loading is slightly outside this range at 1.5 g/L.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
In a dynamic flow-through laboratory study, the bioconcentration potential was determined in bluegill sunfish (*Lepomis macrochirus*). A flow-through proportional diluter system was used to maintain a mean measured water concentration of 12 ± 0.7 mg ^{14}C glyphosate/L for a 35-day exposure period. Subsequently, the fish were exposed for 21-days to flowing uncontaminated well water. During the uptake phase, water was sampled on day 0 and then water and fish were sampled after 2 and 6 hours,

and after 1, 3, 7, 14, 21, 28 and 35 days. During the depuration period, water and fish were sampled on day 1, 3, 7, 10, 14 and 21 (corresponding to day 36, 38, 42, 45, 49 and 56 after test initiation).
Five fish per sampling date were collected from each replicate and pooled into control and treated samples. Six of the control and treated fish were dissected into fillet/edible (body muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). Four fish of the control and treated samples per sampling date were used for whole fish analysis. For metabolite characterisation, 12 fish from the control and treatment group from each aquarium were sampled and dissected on days 7, 14, 21 and 28 of the uptake phase.
The daily bioconcentration factor ranged from <0.11 to 0.38 for fillet, from <0.11 to 0.52 for whole fish, and from <0.11 to 0.63 for viscera, respectively. Uptake tissue concentrations of ^{14}C-glyphosate ranged from <1.4 to 4.6 mg a.s./kg for fillet, from <1.3 to 6.2 mg a.s./kg for whole fish, and from <1.3 to 7.6 mg a.s./kg for viscera, respectively. ^{14}C-residue levels were below minimum quantifiable limits until day 21 for fillet and day 7 for whole fish and viscera samples. Radio-analysis on day 21 of the depuration period indicated 35%, 52% and 51% depuration from fillet, whole fish and viscera, respectively.
The uptake rate constant (K_1) of ^{14}C glyphosate was estimated to be 0.022 ± 0.004 mg a.a./kg in fish/mg/L per day while the depuration rate constant (K_2) was of 0.020 ± 0.01/day. The 50% clearance was estimated to be to 35 ± 18 days.
All validity criteria according to the OECD guideline 305 were fulfilled.

In a flow-through dynamic uptake study of ^{14}C-glyphosate (12 mg a.s./L) by *Lepomis macrochirus*, the time to reach 90% of steady state was estimated to be 120 ± 59 days. The bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61 by the author who considered this study as valid.
RMS considersthat no BCF can be set. However, the results are considered informative and provide evidence that the potential for bioaccumulation of glyphosate is low.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	^{14}C glyphosate (N-phosphonomethylglycine-methyl-^{14}C)
Description:	White powder
Lot/Batch #:	C-1106-4; C-1106-5 (FJGT-07-000)
Purity:	99.2%

2. Vehicle of test material/media: deionised water

3. Test organism:

Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	juvenile
Size:	Length: 6.3 ± 0.18 cm
Body weight:	8.1 ± 0.9 g
Loading:	1 specimen/0.6 L (1.5g fish/L)
Source:	[redacted]
Diet/Food:	Daily with Zeigler Brothers #l Salmon Starter equivalent to approximately 3 % of fish body weight.
Acclimation period:	> 14 days

4. Environmental conditions:

Temperature:	22 ± 1°C
Photoperiod:	16/8 hours light/dark

pH:	7.8 – 8.2
Dissolved oxygen:	6.4 – 8.4 mg/L (76 - 100% of oxygen saturation)
Conductivity:	480 – 540 μS/cm³
Hardness:	238 – 278 $CaCO_3$/L.

5. Dates of experimental work: January 26th to March 22nd 1988

B. STUDY DESIGN

Experimental treatments:

Based on the results of a range-finding test, a 56-days laboratory bioconcentration study of bluegill sunfish (*Lepomis macrochirus*) exposed to glyphosate was conducted using a nominal test concentration of 12 mg ^{14}C glyphosate/L under flow-through conditions. The test was conducted in glass aquaria containing 70 L test solution. A modified proportional diluter system (Hamilton Model 420 dual syringe dispenser), was used for intermittent introduction of test item and water solution at an average rate of 340 mL/min., replacing test volume approximately 7 times/day.

The uptake phase (day 0 – 35) was initiated by transferring groups of 110 specimens to each replicate. Water was sampled on day 0 and water and fish were sampled 0.17 (2 – 6 hours), 1, 3, 7, 14, 21, 28 and 35 of the uptake phase and on day 1, 3, 7, 10, 14 and 21 of the depuration period (corresponding to day 36, 38, 42, 45, 49 and 56 after test initiation) and radio-assayed.

All measurements of radioactivity were made using either a Searle Model Delta 300® Liquid Scintillation Counting (LSC) System or a TM Analytic Model Delta 300® LSC System optimized for carbon-14 sample analysis.

Observations: On sampling days, five fish from each chamber were collected and pooled into control and treated samples total of ten fish each for control and treated). Six of the pooled fish were dissected into fillet/edible (body muscle, skin and skeleton) and viscera/non-edible (fins, head and internal organs). The remaining four fish of the pooled control and treated samples were reserved for whole fish analysis. Additional fish (12 fish from the control and treatment group) were collected and dissected for metabolite characterization on days 7, 14, 21 and 28 of the uptake phase.

Analytical procedures: The levels of ^{14}C-activity calculated as concentrations of ^{14}C-glyphosate in whole fish, fillet and viscera samples were determined by triplicate analysis of homogenised samples using sample combustion followed by liquid scintillation counting.

Statistical calculations: A non-linear kinetic modelling computer program (Dow BIOFAC) was used to determine the uptake rate constant (K_1) and depuration rate constant (K_2). The Bioconcentration factors for the uptake period were determined by dividing the ^{14}C-glyphosate concentration in tissue by the mean ^{14}C-glyphosate concentration in water for corresponding exposure time.

II. RESULTS AND DISCUSSION

A. FINDINGS

Initial water concentrations are shown below, throughout the 35-day study water concentrations ranged from 11 to 13 mg ^{14}C/L, equivalent to 91.7% and 108.3% of the nominal test concentration respectively.

Table B.9.2.2.3-1: Initial water concentrations – Radiochemical/HPLC analysis

Glyphosate in water [mg a.s./L]	% in final concentrate	% Glyphosate	% AMPA
12.3	74.3	95	1.2
12.5	97.8	95.9	1.9
13.2	82.9	95.8	1.8
12.3	85.6	96.6	1.1

Total ^{14}C-radioactivity calculated as ^{14}C-glyphosate in test water and fish tissue during 35 days exposure and 21 days depuration with bluegill sunfish is given below.

Table B.9.2.2.3-2: Summary of results

Parameter	Endpoints
K_1, Uptake rate constant [ppm fish/ppm water/day]	0.022 ± 0.004
K_2, Depuration rate constant [/ day]	0.020 ± 0.010
50% Depuration [days]	35 ± 18
90% Steady-State [days]	120 ± 59
Bioconcentration factor	1.1 ± 0.61
Symptoms	none

Table B.9.2.2.3-3: Total ^{14}C-radioactivity calculated as ^{14}C-glyphosate in test water and bluegill sunfish tissue

	Fillet		Whole fish		Viscera	
Days ↓	[mg a.s./kg]	BCF	[mg a.s./kg]	BCF	[mg a.s./kg]	BCF
3	< LOD	< 0.11	< LOD	< 0.11	< LOD	< 0.11
14	< LOD	< 0.11	4.3	0.36	5.1	0.42
21	1.8	0.15	3.9	0.32	7.6	0.63
28	3.6	0.30	6.2	0.52	6.8	0.57
35	4.6	0.38	4.6	0.38	7.2	0.60

LOD: Limit of detection

Table B.9.2.2.3-4: Depuration of total ^{14}C calculated as ^{14}C-glyphosate from bluegill sunfish during a 21-day clearance period

	Fillet			Whole fish			Viscera		
Days ↓	Conc. [mg a.s./kg]	Depuration		Conc. [mg/kg]	Depuration		Conc. [mg/kg]	Depuration	
		Amount depurated [mg a.s./kg]	[%]		Amount depurated [mg a.s./kg]	[%]		Amount depurated [mg a.s./kg]	[%]
0	4.6	0	0	4.6	0	0	7.2	0	0
1	2.7	1.9	41	13	0	0	5.2	2.0	28
3	2.8	1.8	39	4.1	0.5	11	5.6	1.6	22
7	4.8	0	0	10	0	0	6.2	1.0	14
10	2.1	2.5	54	6.8	0	0	3.4	3.8	53
14	3.0	1.6	35	2.5	2.1	46	3.9	3.3	46
21	3.0	1.6	35	2.2	2.4	52	3.5	3.7	57

B. OBSERVATIONS

Due to the nature of the test compound, a steady-state plateau was never achieved during the 35 days of uptake. No mortality or abnormal behaviour was observed during the conduct of this study. All validity criteria according to the OECD guideline 305 were fulfilled as the temperature variation was < 2°C and the concentration of dissolved oxygen was ≥ 60% saturation. The concentration of the test substance in the chambers was maintained within ± 20% of the mean of measured values during uptake phase and no mortality or abnormal behaviour was observed during the conduct of this study.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a flow-through dynamic uptake study of ^{14}C-glyphosate by Bluegill sunfish (*Lepomis macrochirus)*, the time to reach 90% of steady state was estimated to be 120 ± 59 days. The bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61.

This flow through dynamic uptake study of ^{14}C-glyphosate by Bluegill sunfish (*Lepomis macrochirus)* is considered valid and the bioconcentration factor (BCF) was estimated to be 1.1 ± 0.61 and can be used in risk assessment.

Assessment and conclusion by RMS:
The conclusions reported above by the applicant are not complete. The study authors also reported that:
Radioanalysis on day 21 of the depuration period indicated that 35, 52 and 51% of the 14C-residues had been eliminated for the fillet, whole fish and viscera, respectively. Water samples from treatment days 1, 28, and 35 of the bioconcentration phase were analyzed by HPLC and found to contain 95-97% glyphosate with 1.1-1.9% chromatographing as aminomethylphosphonic acid (AMPA).

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was conducted long before the revised OECD 305 guideline was published. Since then, experience has shown that biological factors such as growth and fish lipid content can have a strong impact on the results and may need to be taken into account (as recommended in the actual guideline).
RMS highlights that:

- fish lipid content was not measured.
- BCF_k s may have not been corrected for growth dilution. (This was not done/reported in this study).

RMS notes that a steady-state could not be observed (concentrations in fish still increasing at the end of the uptake phase). However in view of the very low concentrations levels in fish tissues and the slow increase, RMS is of the opinion that this would have led to an impractically long uptake phase to reach steady-state, so a kinetic approach is preferred in such a case (as it was proposed in this study).

The OECD 305 recommends the use of reference substances of known bioconcentration potential and low metabolism to verify the experimental procedure, when required (e.g. when a laboratory has no previous experience with the test or experimental conditions have been changed). No data was reported in the study report.

Fish loading range of 0.1 g – 1.0g/L is recommended in the guideline. Actual loading was of 1.5 g/L. RMS considers this is acceptable as higher fish-to-water loading rates can be used if it is shown that

the required concentration of test substance was maintained within ± 20% limits, and that the concentration of dissolved oxygen did not fall below 60% saturation (these 2 criteria were fulfilled).

Only one concentration was tested. The test guideline was originally designed for non-polar organic substances. For this type of substance, the exposure of fish to a single concentration was expected to be sufficient, as no concentration effects are expected. However the guideline hence states that : "*if substances outside this domain are tested, or other indications of possible concentration dependence are known, the test should be run with two or more concentrations. If only one concentration is tested, justification for the use of one concentration should be given*". Glyphosate is a polar compound. Therefore, more than one concentrations should have been tested.

The concentration tested was of 12 mg/L.
The guideline recommends that "*the concentration(s) of the test substance should be selected to be below its chronic effect level or 1% of its acute asymptotic LC50, within an environmentally relevant range…*". Based on current knowledge on the substance, RMS considers that the tested concentration is too high.

Overall RMS considers that the study is not robust enough to derive an endpoint. However in view of low lipophilicity of the substance (Log Pow= -6.28 at 25°C at pH 7), a bioaccumulation study is not required.

RMS nevertheless considers that (based on this study) the potential for bioaccumulation of glyphosate is low.

No BCF can be set. However, the results are considered informative and provide evidence that the potential for bioaccumulation of glyphosate is low.

B.9.2.3. Potential for endocrine disruption

A 'Fish short term reproduction assay' (FSTRA; OECD TG 229) has been performed and is summarised thereafter.

Data point:	CA 8.2.3/001
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Fish Short-Term Reproduction Assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*)
Report No	707A-102A
Document No	-
Guidelines followed in study	OECD Guideline 229 (2009) OPPTS/OCSPP Guideline 890.1350 (2009)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 229 (2012):* *Minor:* *- Temperature variation was greater than 2°C for a short time period (< 24 hours).*
Previous evaluation	Yes, EFSA ED Conclusion (2017)[1]
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The 21-day short-term reproduction assay of MON 77973 (glyphosate acid) with the fathead minnow (*Pimephales promelas*) was conducted under flow-through conditions to determine the impact of glyphosate acid on the hypothalamus-pituitary-gonadal (HPG) endocrine axis by evaluating effects on the reproductive system, such as fecundity, fertility, secondary sexual characteristics (tubercles and fatpad scores), gonadosomatic index (GSI) histopathology of gonads as well as plasma vitellogenin. Four groups of adult males and females (2 males and 4 females in each group), were exposed to glyphosate acid at nominal concentrations of 0 (negative control), 0.048, 0.24, 1.2, 6.0, and 30 mg a.s./L, (the highest test concentration was based on one-third of a 96-hr LC_{50} value of a previous acute toxicity test) with a total of 24 fish exposed per treatment and control group. Following a pre-exposure period of 19 days, groups of actively spawning fish, were exposed to glyphosate acid according to the aforementioned treatment groups, for a 21-day exposure period, with survival, fecundity, fertility and general observations recorded daily. The remaining reproductive endpoints were evaluated at test termination, along with fish lengths and fish weights.

The overall arithmetic mean measured glyphosate acid concentrations were (negative control; <LOQ), 0.046, 0.23, 1.2, 6.2, and 33 mg a.s./L, respectively. All performance criteria were met for this study, except for a slight deviation in temperature. Recorded temperatures exceeded the recommended range (25 ± 1 °C), for less than 24 hours on Day 7 when the maximum recorded temperature reached 29.1°C (range of 28.6 - 29.1°C); deviation occurred in three replicates each in the 1.2 mg a.s./L and 6.2 mg a.s./L groups). This deviation was due to a loose wiring between the temperature probe and the heat plates beneath these replicates, which was quickly rectified. Temperature measurements repeated on Day 7, across all affected replicates fell within a 24.4 to 24.7 ºC range. This minor deviation is not considered to have had any impact on study integrity.

There was 100% fish survival in the negative control, 0.046, 0.23, 6.2, and 33 mg a.s./L treatment groups with 91.7% survival in the 1.2 mg a.e./L treatment group.

[1] EFSA (European Food Safety Authority), 2017. Conclusion on the peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate. EFSA Journal 2017;15(9):4979, 20 pp. https://doi.org/10.2903/j.efsa.2017.4979

Glyphosate acid did not result in any significant increases or decreases in weight or length for either sex at any treatment level. There were no observed effects on secondary sex characteristics or clinical signs (i.e., behavioral and other sub-lethal effects) in males or females in any treatment group. The mean number of eggs per female reproductive day in the negative control was 23.5 eggs/day (range: 23.2-23.9 eggs/female/day); fertilization success in the negative control was 97.3%. Fecundity and fertilization success were not significantly different from the negative control for any treatment group.
There were no effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate acid for 21 days. Based on the endpoints evaluated, glyphosate acid is concluded to not impact the function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnows. The study is considered valid.

Table B.9.2.3-1: Summary of FSTRA Findings

Treatment (mg a.e./L) [mean-measured]	Fecundity	Fertilization Success	Tubercle Score		GSI		Gonadal Histopathology		Plasma VTG	
			M	F	M	F	M	F	M	F
0.046	No	No	No	No	No	No	No	No	No	No
0.23	No	No	No	No	No	No	No	No	No	No
1.2	No	No	No	No	No	No	No	No	No	No
6.2	No	No	No	No	No	No	No	No	No	No
33	No	No	No	No	No	No	No	No	No	No

F = Female; GSI = Gonado-Somatic Index; M = Male; VTG = Vitellogenin

The fish short-term reproduction assay (FSTRA) with breeding groups of fathead minnow (*Pimephales promelas*) exposed to glyphosate acid is considered valid. The overall NOEC was 33 mg a.s./L (arithmetic mean measured).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 77973 (glyphosate acid)
Description:	White powder
Lot/Batch #:	GLP-1103-21149-T
Purity:	85.14% before drying (95.93% glyphosate acid, dried)
CAS #:	1071-83-6
Stability of test compound:	Stable. Mean-measured concentrations yielded recoveries of 96-110% of nominal.

2. Vehicle of test material/media: dilution water (filtered well water)

3. Test organism:

Species/sex:	Fathead minnow (*Pimephales promelas*)
Strain:	Not specified
Age at start of dosing:	5.5 months
Weight at start of dosing:	0.9 g (females) – 1.6 g (males)
Source:	[redacted]

Acclimation period:	2 months, plus 19-day pre-exposure period
Diet:	Commercial flake food (Sera Vipan, Sera North America) supplemented with shrimp brine nauplii (Brine Shrimp Direct, Ogden, UT, USA), 2 times/day
Housing:	
Exposure System:	Continuous flow-through diluter system
Flow-through Rate:	44 mL/min
Exposure Vessel:	12 L Glass Aquaria (10 L fill volume)
Spawning Substrate Material:	Inverted semi-circular PVC pipe section (~10 cm)
Source of dilution water:	Fresh filtered and sterilized well water (0.45 µm)
4. Environmental conditions:	
Temperature:	25.4°C (24.3°C – 29.1°C) A minor temperature deviation occurred on Day 7 due to loose wiring between a temperature probe and heat plates beneath replicates B, C and D of the 1.2 mg a.s./L treatment group and replicates A, B and C of the 6.2 mg a.s./L treatment group, with a maximum temperature of 29.1 ºC recorded. The wiring was reattached, and measurements were re-taken later on Day 7. The second measurements in Replicates B, C and D of the 1.2 mg a.s./L and Replicates A, B and C of the 6.2 mg a.s./L treatment groups respectively were 24.7, 24.6, 24.5, 24.4, 24.4 and 24.5ºC, respectively.
pH:	8.1 (8.0 – 8.3)
Dissolved Oxygen	7.2 mg/L (6.0 – 7.9 mg/L)
Total Alkalinity:	173.5 mg/L as $CaCO_3$ (166 – 180 mg/L as $CaCO_3$)
Hardness:	144.5 mg/L as $CaCO_3$ (140 – 148 mg/L as $CaCO_3$)
Photoperiod:	16 h light/ 8 h dark (30-minute transition of low light between light and dark periods).
Light Intensity at Water's Surface:	Mean = 1170 ± 412 lux (range 450 – 1976 lux)
5. Dates of experimental work:	17th October 2011 to 11th January 2012

B. STUDY DESIGN

Experimental treatments

A 14-day range-finding test was conducted at 1.9, 3.8, 7.5, 15 and 30 mg a.s./L, for 14 days, the highest concentration tested, being based on the results of a 96 hour acute toxicity study[2], being approximately one-third of the achieved LC_{50}. In the range-finding test, one incidental mortality occurred at 15 mg a.s./L, with no other signs of toxicity observed in any control or treatment group throughout the test duration.

The definitive test concentration range was 0.048, 0.24, 1.2, 6.0 and 30 mg a.s./L, conducted under flow-through exposure conditions. A nominal stock solution of 225 mg a.s./L – corrected for purity, was pumped into mixing chambers according to treatment group at rates (mL/min) required to achieve the

[2] EG & G Bionomics. 1975. Chronic toxicity of glyphosate to the fathead minnow (*Pimephales promelas* Rafinesque). Monsanto unpublished study BN-75-129. MRID 108171.

final required test concentrations. Test solutions were then pumped into the test chambers (12-L glass aquaria) filled with approximately 10 L of test water. The volume in the test chambers was maintained by an overflow port on one end of each chamber. Into each chamber, a spawning substrate or tile was placed into each chamber. A tile consisted of a semi-circular section of PVC pipe approximately 10 cm in length.

Four replicates were used in each treatment group (including the control group); each replicate consisted of two males and four females, except the fourth replicate at 33 mg a.s./L, where there were three males and three females due to a mis-sexed fish at pre-exposure allocation. Water samples were collected from two alternating replicate test chambers in each treatment and control group for concentration analysis on Days 0, 7, 14, and 21. The limit of quantification (LOQ) was 0.0300 mg a.s./L.

Nominal and arithmetic mean measured glyphosate acid concentrations can be found in the table below.

Table B.9.2.3-2: Summary of Treatment Concentrations in the FSTRA with Glyphosate acid

Treatment ID	Nominal Concentration (mg a.e./L)	Measured Concentration (mg a.e./L)	Mean CV (%)
Negative Control	0.00	< LOQ	NA
Treatment 1	0.048	0.046	9.0
Treatment 2	0.24	0.23	9.9
Treatment 3	1.2	1.2	2.3
Treatment 4	6.0	6.2	2.4
Treatment 5	30	33	7.8

CV = Coefficient of variation; LOQ = Limit of Quantification (0.0300 mg a.e./L)

Observations:

Mortality, Clinical Signs: Survival and general observations were made daily during the 21-day exposure period. External abnormalities and abnormal behavior were noted if observed. Dead fish were removed as soon as possible but were not replaced in either the control or treatment test chambers.

Body Weight and Length: The wet weight and total length of each fish was recorded at test termination. Fish were blotted dry and weighed to the nearest 0.1 mg. Total length was measured to the nearest millimeter.

Secondary Sex Characteristics: Detailed observations of secondary sex characteristics including pigmentation patterns, tubercles, fatpads, and ovipositors were recorded and the external sex was determined at test termination.

Spawning and Mean Fecundity: Spawning tiles were removed from the test chambers daily and any eggs that were present were counted. Fecundity was calculated as the number of eggs per surviving female per reproductive day per replicate. After eggs were counted, they were evaluated for fertilization success. The number of infertile eggs was counted, and the number of fertile eggs was calculated as the difference between the total number of eggs and the number of infertile eggs on the tile. Fertilization success (%) was calculated as the number of embryos divided by the number of eggs, multiplied by 100.

Plasma Vitellogenin (VTG): At study termination, at least two blood samples were collected from the caudal vein/artery of each fish using heparinized microhematocrit tubes. Male fish were processed before female fish to avoid contamination of VTG samples. After collection, the plasma was separated by centrifugation and transferred to a microcentrifuge tube containing lyophilized protease inhibitor (aprotinin). Analysis for vitellogenin was conducted with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosense Laboratories, Bergen, Norway) using an antibody raised against fathead minnow VTG. The procedures used to collect, prepare and analyze the plasma samples

were based upon methodology provided by the ELISA system manufacturer and those presented by the U.S. EPA.

Plasma Sex Steroid Levels: No plasma sex steroids were measured.

Gonadal Histology and Histopathology: Immediately following blood collection, gonads were fixed *in situ* with Davidson's solution, removed from the abdominal cavity, gently blotted and weighed to the nearest 0.1 mg to determine the gonadosomatic index (GSI = gonad wt/body wt x 100). After weighing, each pair of gonads (right and left) was enclosed in a plastic tissue cassette that was then placed in a container of fixative (Davidson's solution). After at least 24 hours of fixation, the gonads in the cassettes were rinsed with 70% ethanol and placed in neutral-buffered formalin. Gonads were then subjected to routine histological processing, embedded in paraffin, and longitudinally sectioned. At the largest cross-sectional area of the gonads, three step sections (each 4-6 microns thick) were cut at approximately 50-micron intervals and all three sections were mounted on a single glass slide. Slides were stained with hematoxylin and eosin, cover-slipped, and then evaluated by a histopathologist.

Gonadal staging for the male fathead minnow was as follows: 0 = undeveloped, 1 = early spermatogenic,2 = mid-spermatogenic, 3 = late spermatogenic, 4 = spent. Gonadal staging for the female fathead minnow was as follows: 0 = undeveloped, 1 = early development, 2 = mid-development, 3 = late development, 4 = late development/hydrated, 5 = post-ovulatory.

Histomorphologic parameters assessed included relative germ cell numbers, alterations in numbers and sizes of non-germ cells (e.g., testicular interstitial cells and ovarian perifollicular cells), and increased degenerative changes. When appropriate, the pathologist used a scoring system to indicate the severity of these changes and other abnormalities according to the following scale: Grade 0 = not remarkable, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, and Grade 4 = marked. Any changes not amenable to grading were designated as "Present". In addition, the stage of developmental maturity of each gonad pair was indicated according to guideline recommendations.

Analytical procedures: Water samples were collected from two alternating replicate test chambers in each treatment and control group for concentration analysis on Days 0, 7, 14, and 21. Samples were collected from mid-depth at each interval, placed in glass vials, and processed immediately for analysis and analyzed by reverse-phase high performance liquid chromatography (HPLC) using variable wavelength detection set at 500 nm. Chromatographic separations were achieved using a YMC-PACK ODS-AM analytical column (150 mm x 4.6 mm, 3-µm particle size). Fresh calibration standards (range: 0.0300 - 0.300 mg a.s./L) were prepared and analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of glyphosate acid in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation. The limit of quantification (LOQ) was 0.0300 mg a.s./L. Four matrix blank samples were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses.

Statistical calculations: Analyses were performed to evaluate differences between treatment and control groups for each of the following endpoints: survival, wet weight, total length, fecundity, fertility, gonado-somatic index (GSI), vitellogenin (VTG) concentration, tubercle score, gonad developmental stage, and incidence and severity of gonad abnormalities. Measurements of VTG are inherently variable, and boxplots of log transformed VTG values were used to identify potential outliers (Tukey's method) that might need special handling in the analyses. No outliers were excluded from analyses in this study. Unless otherwise noted, replicate test chambers were used as the unit of statistical analysis. Males and females were analysed separately for each endpoint when appropriate. Endpoints were first evaluated for monotonicity. Since the responses for all endpoints except male tubercle scores appeared to be monotonic, a step-down Jonckheere-Terpstra trend test was used to evaluate possible trends in the ranks of replicate means to determine concentration responsive trends among the treatment groups. Dunnett's test was used to evaluate male tubercle scores. Survival was analyzed using Fisher's Exact test, and

histopathology severity scores and stages of individuals were analyzed using step-down Jonckheere-Terpstra trend tests. Statistical tests used to evaluate treatment effects were performed at confidence level of $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Mean measured concentrations of glyphosate acid in test solution samples ranged from 96 to 110% of nominal concentrations.

B. OBSERVATIONS

Mortality, Clinical Signs: No treatment-related effects on survival were observed in any treatment group. There were incidental mortalities of one female and one male fish in the 1.2 mg a.s./L treatment group resulting in an overall survival of 91.7%. Survival was 100% in the remaining treatment groups.
No clinical signs were observed in any males or females in the negative control and treatment groups.

Body Weight and Length: No treatment-related effects were observed on mean body weight or mean length in males or females (see table below).

Table B.9.2.3-3: Body Weight and Length at Test Termination in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Body Weight						Length					
	Males			Females			Males			Females		
	n	Mean [g]	± SD	n	Mean [g]	± SD	n	Mean [mm]	± SD	n	Mean [mm]	± SD
Negative Control	4	2.20	0.462	4	1.14	0.047	4	55	2.8	4	46	0.7
0.046	4	2.17	0.348	4	1.11	0.056	4	53	1.8	4	46	0.9
0.23	4	2.28	0.397	4	1.04	0.069	4	55	3.5	4	45	1.1
1.2	4	2.20	0.185	4	1.12	0.051	4	54	1.6	4	46	0.2
6.2	4	2.15	0.272	4	1.07	0.108	4	53	2.3	4	45	1.3
33	4	2.05	0.209	4	1.13	0.094	4	52	1.0	4	46	0.8

Secondary Sex Characteristics: Overall, there were no treatment-related effects on secondary sex characteristics in males or females in all treatment groups. No treatment-related effects were observed on median tubercle scores. Male nuptial median tubercle scores ranged from 15 at 33 mg a.s./L to 19 at 0.046 and 1.2 mg a.s./L; no nuptial tubercles were observed for females. There were 3 males instead of the recommended 2 due to a mis-sexing error in the fourth replicate of the 33 mg a.s./L treatment group. The median scores are unaffected when this fish is removed from the results.

Spawning and Mean Fecundity: No treatment-related effects were observed on mean fecundity and mean fertilization success (see table below).

Table B.9.2.3-4: Fecundity and Fertilization Success in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Fecundity (Eggs per Female per Reproductive Day)		Fertilization Success (%)	
	Mean	± SD	Mean	± SD
Negative Control	23.5	0.33	97.3	0.4
0.046	29.3	5.3	97.6	1.0
0.23	22.7	5.4	98.4	1.4
1.2	24.9	0.89	96.0	2.7
6.2	28.1	6.4	98.1	1.1

33	23.6	2.2	96.7	2.0

Plasma Vitellogenin (VTG): The mean VTG concentration in males in the negative control, 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L treatment groups was 1.01, 0.77, 1.34, 0.75, 0.39 and 0.33 µg/mL, respectively. There were no statistically significant effects on VTG among males in any treatment group in comparison to the negative control ($p > 0.05$). The mean VTG concentration in females in the negative control, 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L treatment groups was 3191, 2124, 2226, 2195, 1442 and 2142 µg/mL, respectively. There were no statistically significant effects on VTG among females in any treatment group in comparison to the negative control ($p > 0.05$).

Table B.9.2.3-5: Plasma Vitellogenin (VTG) in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Males			Females		
	n	Mean [µg/mL plasma]	± SD	n	Mean [µg/mL plasma]	± SD
Negative Control	4	1.01	1.143	4	3191	1170
0.046	4	0.77	0.312	4	2124	807
0.23	4	1.34	2.068	4	2226	624
1.2	4	0.75	1.240	4	2195	403
6.2	4	0.39	0.368	4	1442	550
33	4	0.33[1]	0.210	4	2142	356

[1] In the fourth replicate of the 33 mg a.s./L treatment group, there were 3 males instead of the recommended 2 due to a mis- sexing error. If this fish is removed from analysis of VTG, the treatment means are very similar as when retained (327 when retained vs. 299 when mis-sexed removed). The values in this table reflect data excluding the mis-sexed male.

Gonadal Histology and Histopathology:
There were no treatment-related effects on GSI (see table below) or on median gonadal staging in males or females.

Table B.9.2.3-6: Gonado-Somatic Index (GSI) in Fathead Minnow (*Pimephales promelas*)

Treatment [mg a.s./L] (mean measured)	Males			Females		
	n	Mean GSI (%)	±SD	n	Mean GSI (%)	±SD
Negative Control	4	1.48	0.218	4	14.7	3.28
0.046	4	1.11	0.202	4	14.4	2.04
0.23	4	1.43	0.393	4	13.1	1.66
1.2	4	1.33	0.087	4	14.0	2.58
6.2	4	1.35	0.324	4	15.5	2.06
33	4	1.51[1]	0.325	4	15.8	3.12

[1] In the fourth replicate of the 33 mg a.s./L treatment group, there were 3 males instead of the recommended 2 due to a mis- sexing error. If this fish is removed from analysis of GSI, the treatment means are very similar as when retained (1.51 when retained vs. 1.52 when removed).

No treatment-related effects in gonadal staging were observed. Testes and ovaries from the five treatment groups showed no changes in gonadal staging or increased abnormalities when compared with the negative control.

There were no treatment-related effects or statistically significant differences observed in the histological evaluations of the testes and ovaries. Testes and ovaries from the five treatment groups showed no changes in gonadal staging or increased abnormalities when compared with the negative control.

Minimal and mild granulomatous inflammation was found in male gonads from the mean-measured 0.046 mg a.s./L treatment group, but these observations were not considered to be treatment-related (see table below). No other male gonadal histopathological observations were made.

Table B.9.2.3-7: Gonadal histopathology in male fathead minnow (*Pimephales promelas*)—Selected parameters as discussed above

Treatment (mg a.e./L) [mean-measured]	Severity	Granulomatous Inflammation	
		n	Incidence
Negative Control	0	8	8
	1	8	0
	2	8	0
	3	8	0
	4	8	0
0.046	0	8	4
	1	8	1
	2	8	3
	3	8	0
	4	8	0
0.23	0	8	8
	1	8	0
	2	8	0
	3	8	0
	4	8	0
1.2	0	7	7
	1	7	0
	2	7	0
	3	7	0
	4	7	0
6.2	0	8	8
	1	8	0
	2	8	0
	3	8	0
	4	8	0
33[1]	0	9	9
	1	9	0
	2	9	0
	3	9	0
	4	9	0

[1] In the fourth replicate of the 33 mg a.e./L treatment group, there were 3 males instead of the recommended 2 due to a mis-sexing error. Because the mis-sexed male in the fourth replicate of the mean-measured 33 mg a.e./L treatment group was not explicitly identified, it was included in the histopathologic evaluations.

Mild increased oocyte atresia in females was observed in the negative control, low, and mid concentration treatments, and a single incident of moderate increased oocyte atresia was noted in the high concentration treatment group (see table below). Moderate to marked increases in mature oocytes were observed in two, five, and one females in the negative control and mean-measured 1.2 and 33 mg a.s./L treatment groups, respectively, and were therefore not considered to be treatment-related. Mild granulomatous inflammation was noted in a single female in the negative control and mean-measured 6.2 mg a.s./L treatment group and therefore was not considered to be treatment-related (see table below).

Table B.9.2.3-8: Gonadal histopathology in female fathead minnow (*Pimephales promelas*)—Selected parameters as discussed above

Treatment (mg a.e./L) [mean-measured]	Severity	Increased Ooctye Atresia		Granulomatous Inflammation		Increased Mature Oocytes	
		n	Incidence	n	Incidence	n	Incidence
Negative Control	0	16	15	16	15	16	14
	1	16	0	16	0	16	0
	2	16	1	16	1	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	2
0.046	0	16	15	16	16	16	16
	1	16	0	16	0	16	0
	2	16	1	16	0	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	0
0.23	0	16	16	16	16	16	16
	1	16	0	16	0	16	0
	2	16	0	16	0	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	0
1.2	0	15	13	15	15	15	10
	1	15	0	15	0	15	0
	2	15	2	15	0	15	0
	3	15	0	15	0	15	1
	4	15	0	15	0	15	4
6.2	0	16	16	16	15	16	16
	1	16	0	16	0	16	0
	2	16	0	16	1	16	0
	3	16	0	16	0	16	0
	4	16	0	16	0	16	0
33	0	15	14	15	15	15	14
	1	15	0	15	0	15	0
	2	15	0	15	0	15	0
	3	15	1	15	0	15	0
	4	15	0	15	0	15	1

Following point is a minor deviation from guideline OECD 229 (2012):
- Temperature range was greater than the recommended range (25 ± 2°C) for a short time period (< 24 hours).
This deviation did not have any adverse impact on the study.

The test is regarded as valid, since criteria for test acceptability according to OECD 229 guideline (2012) were met:

- The dissolved oxygen concentration was at least 60% of the air-saturation value throughout the exposure period.
- Water temperature did not differ by more than 1.5ºC between test vessels at any one time during the exposure period and was maintained within ±2ºC of the 25ºC temperature specified, except on Day 7 of the test when the maximum temperature was 29.1ºC for a short duration (< 24 hours). This deviation did not have any adverse impact on the study
- There was more than 90% survival of control animals over the duration of the chemical exposure.
- Mean measured concentrations of the test substance remained within an acceptable range throughout the test (CV < 20%)

III. CONCLUSIONS

Assessment and conclusion by applicant:

Breeding groups of fathead minnows (*Pimephales promelas*) were exposed to glyphosate acid at arithmetic mean measured concentrations of 0.046, 0.23, 1.2, 6.2 and 33 mg a.s./L for 21 days. The endpoints evaluated were adult survival, body length and wet weight, fecundity (cumulative egg production and eggs per female reproductive day), fertilization success, secondary sex characteristics (including fatpad and tubercle scores), GSI, VTG and gonad histopathology. There were no effects on survival, growth, reproduction, secondary sex characteristics, GSI, VTG or gonad histopathology in male or female fish exposed to glyphosate acid for 21 days. Based on the endpoints evaluated, glyphosate acid is concluded to not affect the function of the hypothalamus-pituitary-gonadal (HPG) endocrine axis in fathead minnows.

The fish short-term reproduction assay (FSTRA) with breeding groups of fathead minnow (*Pimephales promelas*) exposed to glyphosate acid is considered valid and the overall NOEC ≥33 mg a.s./L (arithmetic mean measured) can be used for ecotoxicological risk assessment.

Assessment and conclusion by RMS:

This study was performed according to OECD 229 and was part of the follow-up assessment to the existing EFSA Conclusion on the peer review for the renewal of the approval of glyphosate (EFSA Journal 2017;15(9):4979) focussed on the outstanding issues identified in relation to the potential endocrine activity of glyphosate.

Then, no reassessment of this study was deemed necessary by RMS as it is conducted in accordance with the recommended guideline (OECD 229) and its reliability was discussed and agreed by MSs (EFSA Journal 2017;15(9):4979).

The conclusion that was agreed during the peer-review is reported below:

"In the fish short-term reproduction study, reduced vitellogenin levels were observed. These differences were not statistically significant. None of the reproductive parameters (fecundity, fertilization success, gonadosomatic index, gonad histology) were affected. In case of an endocrine mode of action, it would be expected to detect reproductive effects in this study".

NOEC = 33 mg glyphosate acid/L (mean measured)

B.9.2.4. Acute toxicity to aquatic invertebrates

B.9.2.4.1. Acute toxicity to Daphnia magna

Data point:	CA 8.2.4.1/001
Report author	[redacted]
Report year	2003
Report title	MON 78623: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*)
Report No	139A-309
Document No	-

Guidelines followed in study	OECD Guideline 202 (1984) OPPTS 850.1010 (1996) EU Directive 67/548/EEC Method C2 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004):* *Minor:* *- Immobilisation was recorded after 19 h of exposure (this is in addition to the guideline requirement).*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of Mon 78623 (Glyphosate K-Salt) on *Daphnia magna* were evaluated in a 48-hour static toxicity test performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg test item/L, equivalent to 74, 149, 298, 596 and 1193 mg glyphosate acid equivalents/L. These nominal concentrations are equivalent to mean measured concentrations of 165, 312, 624, 1285 and 2582 mg test item/L. In addition, a negative control group (well water only) was run in parallel. There were two vessels prepared for the control and for each treatment, each containing ten daphnids.

The total number of immobile *Daphnia magna* was recorded at 19 h, 24 h and 48 h after test initiation. Mean measured concentrations were recorded at the beginning and at the end of the tests.

Mean overall measured concentrations of glyphosate (acid equivalents) ranged between 100 and 106% of the nominal values. Glyphosate K-salt was not detected in the control group. At 624 mg test item/L 65% of the daphnids were observed to be lethargic at the bottom of the test chamber at test termination. Immobility at 48 h at concentrations of 1285 and 2582 mg test item/L were 5 and 25%, respectively and all remaining daphnids at these two test concentrations were lethargic at the bottom of the test chamber. All validity criteria according to the guideline OECD 202 were fulfilled.

In conclusion, the 48 h EC_{50} for *Daphnia magna* exposed to Glyphosate K-salt was calculated to be > 2582 mg/L, equivalent to >1231.6 mg glyphosate acid/L based on mean measured concentrations. The 48- hour no-effect level (NOEC) for Glyphosate K-salt was determined to be 312 mg/L, equivalent to 148.8 mg glyphosate acid/L based on mean measured concentrations. The study is considered to be valid. Based on lethargy, RAR 2015 recalculated EC_{50} to be 278 mg a.e./L and NOEC to be 149 mg a.e./L, arithmetic mean measured.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 78623 (Glyphosate K-salt)
Active substance	Glyphosate acid
Description:	Yellow liquid
Lot/Batch #:	GLP-0108-11688-F

Purity:	47.7% acid equivalents
2. Vehicle of test material/media:	Well water
3. Test organism:	
Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	2 × 10 specimens for 250 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None
4. Environmental conditions:	
Temperature:	19.5 – 20.0°C
Photoperiod:	16 hours light / 8 hours dark with 30 min transition period
pH:	5.7 – 8.1 (test item) 8.1 – 8.2 (control)
Dissolved oxygen:	≥ 8.6 mg/L (≥ 96% saturation)
Conductivity:	310 µmhos/cm
Hardness:	140 mg $CaCO_3$/L.
Alkalinity:	184 mg $CaCO_3$/L
5. Experimental dates:	December 3, 2002 to December5, 2002

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, a definitive toxicity test was performed using nominal concentrations of 156, 313, 625, 1250 and 2500 mg test item/L (mean 165, 312, 624, 1285 and 2582 mg test item/L in a static test setup. The test solutions were prepared using test facility well water (Dissolved oxygen ≥ 96%, pH = 5.7 – 8.1, hardness 140 mg $CaCO_3$/L.). In addition, a control group was exposed to well water (negative control). There were two replicates per treatment, each containing ten daphnids. Test chambers were 250 mL glass beakers containing approx. 250 mL of test medium.

2. Observations: Total number of immobile *Daphnia magna* was recorded at 19h, 24 h and 48 h after the test initiation. Temperature of the test solutions was measured at the test initiation and termination. Hardness, alkalinity and specific conductance of the dilution water were measured at test initiation. The pH value and oxygen saturation were measured at test initiation and at 24h and 48h. For analysis of test substance concentration with HPLC, test medium was collected from the replicate test chambers at 0 and 48 h.

The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 per cent of the daphnids should have been immobilised or show or other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

3. Statistical calculations: Since the immobility was < 50%, no statistical calculation of EC_{50} values was possible. Therefore, EC_{50} and NOEC values were determined by visual inspection.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analytics confirm the stability of the test substance, since the recovery was 99 – 105% at test start and 97 – 107% at test end. Results are based on arithmetic mean measured concentrations.

Table B.9.2.4.1-1: Analytical results

Nominal concentration MON 78623 [mg/L]	**Control**	**156**	**313**	**625**	**1250**	**2500**
0 h mean measured concentration [mg/L]		163	311	636	1279	2548
48 h mean measured concentration [mg/L]		167	314	612	1291	2616
Mean measured over 48 h Glyphosate K-salt (MON 78623) [mg/L]		165	312	624	1285	2582
% of nominal		106	100	100	103	103
Mean Measured over 48 h Glyphosate acid [mg/L]	-	78.7	148.8	297.6	612.9	1231.6

The EC_{50} and NOEC are based on mean measured concentrations of 165, 312, 624, 1285 and 2582 mg test item/L and are given below.

Table B.9.2.4.1-2: Endpoints

Endpoints	**Glyphosate K-salt [mg/L]**	**Glyphosate Acid [mg a.e./L]**
48 h EC_{50}	> 2582	> 1231.6
NOEC	312	148.8

B. OBSERVATIONS

In the negative control and at mean measured concentrations of 165 and 312 mg test item/L no effects were observed. At 624 mg test item/L 65% of the daphnids were observed to be lethargic at the bottom of the test chamber at test termination. Immobility at 48 h at 1285 and 2582 mg test item/L was 5 and 25%, respectively. All remaining daphnids were lethargic at the bottom of the test chamber.

Table B.9.2.4.1-3: Lethal effects of glyphosate K-salt to *Daphnia magna*

Mean measured Glyphosate K-salt (MON 78623) [mg/L]	**Control**	**165**	**312**	**624**	**1285**	**2582**
Mean Measured Glyphosate acid [mg a.e./L]	**-**	**78.7**	**148.8**	**297.6**	**612.9**	**1231.6**
Immobility (19 h) [%]	0	0	0	0	0	0
Immobility (24 h) [%]	0	0	0	0	0 (8C)	0 (17C)
Immobility (48 h) [%]	0	0	0	0 (13C+G)	1 (19C+G)	5 (15C+G)

C = lethargic; G = on bottom of test chamber; AN = appear normal

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 h EC_{50} for *Daphnia magna* exposed to Glyphosate K-salt was calculated to be > 2582 mg/L, equivalent to >1231.6 mg a.e./L based on mean measured concentrations. The 48- hour no-effect level (NOEC) for Glyphosate K-salt was determined to be 312 mg/L, equivalent to 148.8 mg a.e./L based on arithmetic mean measured concentrations.

Based on lethargy, RAR 2015 recalculated EC_{50} to be 278 mg a.e./L and NOEC to be 149 mg a.e./L, arithmetic mean measured.

The study is considered valid and reliable for the risk assessment of glyphosate.

Assessment and conclusion by RMS:

The study is considered valid (validity criteria are met).

The pH value at the highest tested concentration (pH of 5.7) was slightly below the recommended range of 6-9, this is considered minor deviation.

No toxic reference has been tested. However, this is not a validity criteria of OECD 202 (2004) in which it is indicated that a reference substance 'may' be tested for EC50 as a means of assuring that the test conditions are reliable.

In view of the analytical results, the geometric mean concentrations should have been used instead of arithmetic means. However, RMS checked and found that the mean concentrations are identical based on arithmetic mean or geometric mean. Therefore, the toxicity values would not be changed.

As previously noted in RAR 2015, the observed effects "lethargic" was not defined in the test protocol and were assimilated to immobility.
According to OECD 202, "*those animals that are not able to swim within 15 seconds, after gentle agitation of the test vessel are considered to be immobilised (even if they can still move their antennae)*". Based on this, the EC50 value was recalculated.

RMS still considers the recalculated EC50 (based on lethargy as sign of immobilisation in the sense of the OECD 202 definition) relevant for the risk assessment. Moreover, the study summary indicates that at 624 mg test item/L 65% of the daphnids were observed to be lethargic at the bottom of the test chamber at test termination. Immobility at 48 h at 1285 and 2582 mg test item/L was 5 and 25%, respectively. All remaining daphnids were lethargic at the bottom of the test chamber. Therefore, it cannot be excluded that lethargy is precursory to mortality.

The 48 h EC50 for *Daphnia magna* exposed to glyphosate K-salt was re-calculated to be 592 mg/L, equivalent to 278.24 mg glyphosate acid/L based on mean measured concentrations.
The 48- hour no-effect level (NOEC) for glyphosate K-salt was determined to be 312 mg/L, equivalent to 148.8 mg glyphosate acid/L based on mean measured concentrations.

Data point:	CA 8.2.4.1/002
Report author	[redacted]
Report year	2000
Report title	Acute toxicity of glifosato IPA tecnico [redacted] to *Daphnia magna*
Report No	RF-D51.017/00
Document No	-
Guidelines followed in study	OECD 202 (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the guideline OECD 202 (2004):* *Minor:* *- The concentration of the test substance in the test media was measured only at the beginning of the study.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Summary

The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 100, 180, 320, 560, and 1000 mg a.s./L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control). Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test. The analysed test concentrations ranged between 75.90 and 139.70% of the nominal values. Therefore, the results reported are related to initial measured concentrations of the test item. The 48-h EC50 for *Daphnia magna* exposed to glyphosate isopropylamine salt was greater than 1397 mg/L based on initial measured concentration (corresponding to 471mg glyphosate acid). The NOEC after 48 h based on immobilisation was 1397 mg test item/L (equivalent to 471 mg a.e./L). All validity criteria according to the guideline OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate Isopropylamine Salt
Lot/Batch #:	MJRT 025-201-104
Purity:	612.7 g/kg salt equivalent (analysed on May 02, 2000)

2. Vehicle of test material/media and/or positive control: Vehicle: Water

Positive control: Toxic standard (potassium dichromate)

3. Test organism:

Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (30 mL glass beakers containing 20 mL test solution)
Source:	Carolina Biological Supply Company, Burlington, North Carolina (USA) and maintained as a stock culture at BIOAGRI

4. Environmental conditions:

Temperature:	21.1 to 21.2°C
pH:	Start of the test: 5.56-7.39 End of the test: 5.54-7.81
Dissolved oxygen:	Start of the test: 6.10-6.27 mg O_2/L End of the test: 5.57-5.67 mg O_2/L
Conductivity:	603.0 mg/L µS/cm
Hardness:	248 mg $CaCO_3$
Photoperiod:	Light/dark 0/24 h
5. experimental dates:	June 6th, 2000 to June 15th, 2000

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 100, 180, 320, 560, and 1000 mg a.s./L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control). A reference test using potassium dichromate was carried out in order to verify the sensitivity of the test system. The primary stock solution of nominal concentration of 1000 mg a.s./L was prepared by dissolving 500 mg test item in 500 mL water. Appropriate amounts of this stock solution were diluted to prepare the lower test concentrations of 100, 180, 320, and 560 mg a.s./L. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test.

3. Statistical calculations: The EC_{50} for glyphosate could not be quantified due to the absence of toxicity of the test item, therefore, no statistical analysis was performed. The EC_{50} value for the reference substance potassium dichromate was calculated by applying Trimmed Spearman-Karber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 75.90 and 139.70% of the nominal values. Therefore, the results reported are related to measured concentrations of the test item.

Table B.9.2.4.1-4: Analytical results

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	% of nominal
Control	-	-
100	75.9	75.90
180	150.0	83.33
320	282.8	88.37
560	693.6	123.85
1000	1397	139.70

The EC_{50} value is given below based on initial measured concentrations.

Table B.9.2.4.1-5: Endpoints

Endpoints	Test item mg/L	Glyphosate acid [mg/L]
EC_{50} (48 h)	> 1397	> 471

The reference substance potassium dichromate resulted in a 48-h EC_{50} of 1.22 mg/L (95% CL = 1.12-1.35 mg/L).

B. OBSERVATIONS

After 24 hours and 48 hours of exposure neither in the control nor in the test item concentration vessels immobilisation of *Daphnia* was observed.

The effects of glyphosate on *Daphnia magna* are shown below.

Table B.9.2.4.1-6: Effects of glyphosate on *Daphnia magna*

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Number of immobile *Daphnia* after 48 hours
Control	-	20	0	0
100	75.9	20	0	0
180	150.0	20	0	0
320	282.8	20	0	0
560	693.6	20	0	0
1000	1397	20	0	0

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48-h EC_{50} for *Daphnia magna* exposed to glyphosate isopropylamine Salt was > 1397 mg test item/L (corresponding to 471 mg a.e./L) based on initial measured concentration. The NOEC after 48 h based on immobilisation was ≥ 1397 mg test item/L (corresponding to ≥ 471 mg a.e./L).

All validity criteria according to the OECD 202 were fulfilled, the study is therefore considered valid and the EC_{50} of 471 mg a.e./L and the NOEC of ≥ 471 mg a.e./L can be used in risk assessment.

Assessment and conclusion by RMS:
The study is considered valid (validity criteria are met).

The concentration of the test substance in the test media was measured only at the beginning of the study. Based on the other acute toxicity tests on daphnia for which analytical verifications are available, it could be confirmed that glyphosate is satisfactorily maintained in 48h daphnid test. Thus RMS considers that it is likely that the targeted concentrations were sufficiently maintained during the test duration. (48h). RMS considers the results reliable.

The toxic reference performed well.

The pH value at the highest tested concentration (pH of 5.6) was slightly below the recommended range of 6-9, this is considered minor deviation.

The 48-h EC50 for *Daphnia magna* exposed to glyphosate isopropylamine salt was greater than 1397 mg/L based on initial measured concentration (corresponding to 471mg glyphosate acid). As the highest concentration showed no immobility on the daphnids, the NOEC after 48 h based on immobilisation was 1397 mg a.s./L, equivalent to 471mg glyphosate acid, based on initial measured concentrations.

Data point:	CA 8.2.4.1/003
Report author	[redacted]
Report year	2000
Report title	Acute toxicity of glifosate tecnico [redacted] to *Daphnia magna*
Report No	RF-D51.39/99
Document No	-
Guidelines followed in study	OECD 202 (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the guideline OECD 202 (2004):* *Minor:* • *The concentration of the test substance in the test media was measured only at the beginning of the study.*
Previous evaluation	Yes, accepted in RAR (2015).

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid with restriction (pH issue)

Summary
The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 100, 180, 320, 560, and 1000 mg a.s./L (corresponding to 103.40, 179.56, 334.11, 597.06, and 1051.12 mg a.s./L measured concentrations). In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control).
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test. The analysed test concentrations ranged between 99.75 and 106.61% of the nominal values. The results reported are related to initial measured concentrations of the test item.
The authors concluded that the NOEC after 48 h based on immobilisation was 179.56 mg a.e./L.
The RMS agreed with the proposed NOEC and considered the 48-h EC50 (immobilisation) for *Daphnia magna* exposed to glyphosate to be greater than 334 mg glyphosate acid/L based on initial measured concentrations.
All validity criteria according to the guideline OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	95%

2. Vehicle of test material/media and/or positive control: Vehicle: Water
Positive control: Toxic standard (potassium dichromate)

3. Test organism:

Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (30 mL glass beakers containing 20 mL test solution)
Supplier:	Carolina Biological Supply Company, Burlington, North Carolina (USA) and maintained as a stock culture at BIOAGRI

4. Environmental conditions:

Temperature:	20.2 to 21.5°C
pH:	Start of the test: 3.06-7.40 End of the test: 3.10-7.96
Dissolved oxygen:	Start of the test: 5.7-6.2 mg O_2/L

End of the test: 4.4-4.6 mg O_2/L

Conductivity: 410 mg/L µS/cm

Hardness: 245 mg $CaCO_3$

Photoperiod: Light/dark 0/24 h

5. Experimental dates: October 13th, 1999 to October 28th, 1999

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 100, 180, 320, 560, and 1000 mg a.s./L (corresponding to 103.40, 179.56, 334.11, 597.06, and 1051.12 mg a.s./L measured concentrations). In addition, 4 x 5 *Daphnia* were exposed to test water without test substance (blank control). A reference test using potassium dichromate was carried out in order to verify the sensitivity of the test system. The primary stock solution of nominal concentration of 1000 mg a.s./L was prepared by dissolving 1000 mg test item in 1000 mL water. Appropriate amounts of this stock solution were diluted to prepare the lower test concentrations of 100, 180, 320, and 560 mg a.s./L. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of glyphosate in the test medium were taken from the control and from all test concentrations at the beginning of the test.

3. Statistical calculations: The EC_{50} value for glyphosate and reference substance potassium dichromate was calculated by applying Trimmed Spearman-Karber method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 99.75 and 106.61% of the nominal values. The results reported are related to initial measured concentrations of the test item.

Table B.9.2.4.1-7: Analytical results

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	% of nominal
Control	-	-
100	103.40	103.40
180	179.56	99.75
320	334.11	104.4
560	597.06	106.61
1000	1051.12	105.11

The effects of glyphosate on *Daphnia magna* are shown below.

The 24 and 48 hour EC_{50} values (based on measured concentrations) are given below:

Table B.9.2.4.1-8: Endpoints EC_{50} values for *Daphnia magna*

Time	EC_{50} (mg a.s./L)	95 % confidence interval (mg a.s./L)
24 h	530.42	471.64 - 596.52
48 h	420.59	388.02 – 455.90

The reference substance potassium dichromate resulted in a 48-h EC_{50} of 0.68 mg/L (95% CL = 0.63-0.75 mg/L).

B. OBSERVATIONS

Table B.9.2.4.1-9: Effects of glyphosate on *Daphnia magna*

Nominal concentration [mg test item/L]	Measured concentration [mg test item/L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Immobility after 24 hours [%]	Number of immobile *Daphnia* after 48 hours	Immobility after 48 hours [%]
Control	-	20	0	0	0	0
100	103.40	20	0	0	0	0
180	179.56	20	0	0	0	0
320	334.11	20	0	0	2	10
560	597.06	20	14	70	20	100
1000	1051.12	20	20	100	20	100

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48-h EC_{50} for *Daphnia magna* exposed to glyphosate technical was 420.59 mg a.e./L based on initial measured concentration. The NOEC after 48 h based on immobilisation was 179.56 mg a.e./L.

All validity criteria according to the OECD 202 were fulfilled. The study is therefore considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

The study is considered valid (validity criteria are met).

The concentration of the test substance in the test media was measured only at the beginning of the study. Based on current knowledge on the substance, RMS considers that it is likely that the targeted concentrations were sufficiently maintained during the test duration (48h) (as in CA 8.2.4.1/004 [redacted] (1996), conducted in similar conditions). RMS considers the results reliable.

The toxic reference performed well.

The pH values at the two highest tested concentration (pH of 5.04 at 597 mg/L and 3.06 at 1051 mg/L) were below the recommended range of 6-9. This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of glyphosate acid).
The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6.0-9.0, the stock solution should be adjusted to lie within the specified range of pH 6-9 (OECD 202). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment would have been the most suitable options. However, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the pH should always be in a range required to maintain the health of the organisms tested (here 6-9). pH are within acceptable range up and including 334 mg a.e./L (measured). Thus the endpoint is set considering the three lowest concletrations..

The 48-h EC50 (immobilisation) for *Daphnia magna* exposed to glyphosate was considered to be greater than 334 mg glyphosate acid/L based on initial measured concentrations.
The 48 hour NOEC was calculated to be 179.6 mg glyphosate acid/L.

Data point:	CA 8.2.4.1/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to *Daphnia magna*
Report No	AB0503/C
Document No	-
Guidelines followed in study	OECD 202 (1984). EPA FIFRA, Subdivision E, Guideline 72-2
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 202 (2004): none*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid with restriction (pH issue)

Summary
The effects of glyphosate acid on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 10, 18, 32, 56, 100 and 180 mg/L of glyphosate acid and a pH adjusted 1000 mg/L test concentration of glyphosate acid. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control).

Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and

48 hours thereafter. The concentration of glyphosate acid in the test solutions were measured at 0 and 48 hours.

The analysed test concentrations ranged between 85 and 100% of the nominal values, therefore, the results reported are related to nominal concentrations of the test item. All validity criteria according to the guideline OECD 202 were fulfilled.
The authors concluded that the 48 hour EC_{50} for Daphnia exposed to glyphosate acid falls between 100 and 180 mg/L, where there was zero and 100% immobility, respectively. Using linear interpolation between these two concentrations, the EC_{50} is 136.5 mg/L. The study is considered to be valid.
RMS considered that due to pH issue the EC50 and NOEC should be greater than 100 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%

2. Vehicle of test material/media and/or positive control: Vehicle: Dilution water
Positive control: none

3. Test organism:

Species:	*Daphnia magna* Straus
Age of animals:	Neonates (< 24 h old)
Loading:	5 organisms per vessel (250 mL glass beakers containing 200 mL test solution) which corresponds to 25 *Daphnia*/L.
Source:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	20.5-20.8°C
pH:	4.21-8.98
Dissolved oxygen:	8.7-9.0 mg O_2/L
Conductivity:	693 mg/L µS/cm
Hardness:	263 mg $CaCO_3$
Photoperiod:	16 hours light / 8 hours dark with 20 minute transition periods

5. Experimental dates: July 24th, 1995 to July 26th, 1995

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate acid on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 10, 18, 32, 56, 100 and 180 mg/L of glyphosate acid and a pH adjusted 1000 mg/L test concentration of glyphosate acid. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control).
A stock solution of nominal concentration of 1000 mg a.s./L was prepared by dissolving 1000 mg test item in 1000 mL dilution water. The 10 to 180 mg a.s./L test solutions were prepared by dispersing aliquots of the stock solution to dilution water.
A further 1000 mg a.s./L stock solution was prepared by dissolving 1 g of glyphosate acid in 1 litre of

dilution water. The pH of this stock solution was adjusted from 2.59 to 8.98 using 12 mL of 1 M sodium hydroxide. All stock and test solutions were observed to be clear and colourless. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. The concentrations of glyphosate acid in the test solutions were measured at 0 and 48 hours.

3. Statistical calculations: The EC_{50} values for the 10 and 180 mg a.s./L test concentrations were calculated with the binomial method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analysed test concentrations ranged between 85 and 100% of the nominal values, therefore, the results reported are related to nominal concentrations of the test item.
Due to an oversight at 0 hours the pH adjusted 1000 mg a.s./L test solution was not sampled for analysis and therefore a sample was taken at 24 hours. The lack of 0 hour analysis for this concentration was considered not to have affected the validity of the study since analysis at 24 and 48 hours gave results which were close to the nominal value (100 and 83%, respectively).

Table B.9.2.4.1-10: Analytical results

Nominal concentration [mg/L]	Measured concentration of Glyphosate acid [mg/L]		Mean measured concentration of Glyphosate acid [mg/L]	% of nominal
	0 hours	48 hours		
Control	< 0.0039	< 0.0039	< 0.0039	-
10	8.6	8.4	8.5	85
18	16*	16*	16	89
32	29	29	29	91
56	49	49	49	88
100	92	93	93	93
180	180	180	180	100
1000 (pH adjusted)	1000[#]	830	920	92

[*]Triplicate analysis
[#] measured at 24 hours.

The 24 and 48 hour EC_{50} values (based on nominal concentrations of glyphosate acid) are given below.

Table B.9.2.4.1-11: EC_{50} values for *Daphnia magna*

Time	EC_{50} [mg a.s./L]	95 % confidence interval [mg a.s./L]
24 h	130	100-180
48 h	130	100-180

The pH adjusted 24 and 48 hour EC_{50} values (based on nominal concentrations of glyphosate acid) are given below:

Table B.9.2.4.1-12: EC_{50} values for *Daphnia magna* (pH adjusted)

Time	EC_{50} [mg a.s./L]	95 % confidence interval [mg a.s./L]
24 h	>1000	-
48 h	>1000	-

B. OBSERVATIONS

The results obtained from this study indicate that the toxicity of glyphosate acid below 1000 mg/L was caused by pH values less than 5.

The effects of glyphosate acid on *Daphnia magna* are shown below.

Table B.9.2.4.1-13: Effects of glyphosate acid on *Daphnia magna*

Nominal concentration [mg a.s./L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Immobility after 24 hours [%]	Number of immobile *Daphnia* after 48 hours	Immobility after 48 hours [%]
Control	20	0	0	0	0
10	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	0	0
180	20	20	100	20	100
1000 (pH adjusted)	20	0	0	0	0

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Due to 0% mortality at 100 mg/L and 100% mortality at 180 mg a.s./L, the 48 hour EC_{50} for Daphnia exposed to glyphosate acid falls between 100 and 180 mg/L. Using linear interpolation between these two concentrations, the EC_{50} is 136.5 mg/L. The NOEC was 100 mg a.s./L (nominal).

All validity criteria according to the OECD 202 were fulfilled, so the study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

This study is considered valid. The study was already considered in previous assessment (DAR/RAR).

The pH value at 180 mg/L (pH of 4.21) was below the recommended range of 6-9. This deviation was negatively correlated with increasing concentrations indicating that this pH decrease was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The guideline recommends that where the chemical itself causes a change of the pH of the test medium outside the range of pH 6-9, the stock solution should be adjusted to lie within the specified range of pH 6-9 (OECD 202). Nevertheless, it is RMS opinion that to be able to distinguish effects due to the acidification of the media from other effects test with and without adjustment at same concentrations would have been the most suitable options. However, the magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions. Furthermore, according to OECD guidelines, the pH should always be in a range required to maintain the health of the organisms tested (here 6-9). pH are within acceptable range up and including 100 mg a.e./L.
The pH adjusted 48-h EC50 for *Daphnia magna* exposed to glyphosate acid (>1000 mg/L) can not be considered a relevant endpoint in absence of intermediate doses between 100 and 1000 mg/L to have a suitable dose response.

Since no dose response relationship can be estimated from the data (0% and 100 % effect were reported at the two highest concentrations 100 and 180 mg/L), no robust EC50 is calculable. The presented 48-h EC50 (immobilisation, 136.5 mg a.s./L) for Daphnia magna exposed to glyphosate acid is not supported by RMS.

48 hour EC50 for Daphnia exposed to glyphosate acid >100 mg/L.

Data point	CA 8.2.4.1/005
Report author	[redacted]
Report year	1995
Report title	The acute toxicity of glyphosate to *Daphnia magna*
Report No	710/22
Document No	-
Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations	Toxicity of technical glyphosate (purity >94 %) to aquatic organisms (*Daphnia magna*), 48 hours test.
Short description of results	NOEC 24 h = 100 mg a.s./L LC_{50} 24 h >100 mg a.s./L NOEC 48 h = 18 mg a.s./L LC_{50} 48 h = 40 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	These data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015. The study is considered as supportive because the report is not available and therefore it cannot be concluded on the study validity according the current guideline requirements.

Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS:
RMS notes that this study (reference 95-00537) was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was requested to the BVL but could not be provided to RMS.
For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical. The endpoint measured in this study is the lowest acute toxicity endpoint for Daphnia magna.
The endpoint previously used (in RAR 2015) is then reported here and will be used for the risk assessment:
LC50 48 h = 40 mg glyphosate acid./L
NOEC 48 h = 18 mg glyphosate acid./L

Data point:	CA 8.2.4.1/006
Report author	[redacted]
Report year	1995
Report title	Acute Toxicity Study in *Daphnia magna* with Glyfosaat
Report No	141863
Document No	-
Guidelines followed in study	OECD Guideline 202 (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004):* *Minor:* *- Only two replicates* *- Only one test concentration of 100 mg/L.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Summary
The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The toxicity test was performed using three nominal concentrations, 1, 10 and 100 mg test item/L. Furthermore, a blank control was tested. Ten daphnids were exposed to the concentrations of 1 and 10 mg test item/L (in one test vessel for each test concentration). 2 replicates with 10 daphnids each were prepared for the highest test concentration of 100 mg test item/L and the control.

At or below the highest test nominal concentration, no immobilisation was observed in tested daphnids during the 48 h exposure period. Hence, the 48 h EC_{50} for *Daphnia magna* exposed to glyphosate was determined to be > 100 mg a.e./L. All validity criteria according to OECD 202 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96%

2. Vehicle of test material/media and/or positive control: Vehicle: ISO-medium (in milli-RO water)
Positive control: $K_2Cr_2O_7$

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 80 mL test medium
Source:	In-house culture

4. Environmental conditions:

Temperature:	19.5°C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.0 – 8.1 (control), 5.2 - 5.5 (100mg test item/L)
Dissolved oxygen:	8.9 – 9.5 mg O_2/L
Hardness:	250 mg $CaC0_3$/L

5. Experimental dates: April 12, 1995 to April 14, 1995

B: STUDY DESIGN AND METHODS

1. Experimental treatments: A range finding test, which was considered as the final test (since no immobility of daphnids was observed at or below the highest test concentration), was performed using three nominal concentrations, 1, 10 and 100 mg test item/L, prepared using ISO-medium (in milli-RO water). The test was conducted in a static test setup for 48 hours in 100 mL vessels containing 80 mL test solution each. In addition, a control group was exposed to test medium without test substance or other additives. The test consisted of one vessel per treatment (containing 10 daphnids each) for the test concentrations of 1 and 10 mg test item/L and two vessels (containing 10 daphnids each) for the highest test concentration of 100 mg test item/L and for the control.

2. Observations: Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.

The pH-values were measured at test initiation and termination, for all concentrations and the control. The oxygen saturation was measured at test initiation for the control and the highest test concentration and at test termination for all concentrations and control. The temperature was controlled daily in one control vessel, starting from the beginning of the test.

Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis using samples taken at test start (0 h) and test termination (48 h).

3. Statistical calculations: Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed on samples of the highest test concentration. Before introduction of the daphnids 112% of the nominal glyphosate concentration was recovered in the test media. In the aged test media 109% of the nominal concentration was recovered.

As the mean measured content of the test item ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.4.1-14: Analytical results

Nominal Concentration [mg/L]	Time [hours]	Measured	% of nominal
0	0	0	-
0	48	0	-
100	0	112	112
100	48	109	109

The EC_{50} value is given below based on nominal concentrations.

Table B.9.2.4.1-15: Endpoints

Endpoints	Glyphosate [mg a.e./L]
EC_{50} (48 h)	> 100

Reference item: The 48h-EC_{50} for the reference item was 0.52 mg/L (95% CL = 0.50 – 0.55 mg/L), which was within the range of expected responses. Hence, the sensitivity of this batch of D*aphnia magna* was in agreement with the historical data collected at test facility.

B. OBSERVATIONS

At or below the highest test nominal concentration, no immobilisation was observed in tested daphnids during the 48 h exposure time. Also, all validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
Under the conditions of the present test, glyphosate induced no visible effects in *Daphnia magna* at 100 mg a.e./L, the only concentration tested. Hence, the 48 h EC_{50} for *Daphnia magna* exposed to glyphosate was determined to be > 100 mg a.e./L and the NOEC ≥ 100 mg a.e./L. Although this limit test was conducted with only two replicates, all validity criteria according to the OECD 202 were fulfilled. Therefore, the study is considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

Only 10 individuals were tested at 1 and 10 mg/L. 20 individuals (as recommended) were tested at 100 mg/L.
The pH value at 100 mg/L (pH of 5.2) was below the recommended range of 6-9 (due to the test item). However no immobility was observed.
The toxic reference performed well.

This study is considered valid.

48 hour EC50 for Daphnia >100 mg glyphosate acid/L (nominal).

Data point:	CA 8.2.4.1/007
Report author	[redacted]
Report year	1994
Report title	Acute Toxicity in *Daphnia magna*; Test Article: 'Glyphosate isopropylamine salt'
Report No	83-91-0737-00-93
Document No	-
Guidelines followed in study	OECD Guideline 202
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004):* *Minor:* *- Limit test with one concentration (100 mg test item/L)*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability: (RMS)	Valid

Executive Summary

The acute effects of glyphosate isopropylamine salt on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The test was conducted to supplement the results of the acute toxicity test already performed as a range finding study for the 21 d reproduction test in *Daphnia magna* (IBR Project No. 89-91-2328-05-93).

The acute toxicity test was performed under static conditions as limit test using only one test concentration of nominal 100 mg test item/L, equivalent to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate/L. In addition, a control group was exposed to reconstituted water (Elendt-medium). As a toxic reference, daphnids were exposed to 0.4 and 1.4 mg/L of the reference substance $K_2Cr_2O_7$.
There were four test vessels per treatment, each containing five *Daphnia magna* (25 mL volumetric cylinder containing 10 mL test medium).

Temperature, pH-value and oxygen saturation of the test solutions were measured at test initiation and termination. Total number of mobile daphnids and the rate of immobilisation were recorded 24 and 48

h after test initiation. At 100 mg test item/L, none of the *Daphnia magna* was found to be immobilised. The EC_{50} was determined to be >100 mg test item/L, equivalent to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg a.e./L (nominal). All validity criteria according to OECD 202 were fulfilled.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C

2. positive control: 0.4 and 1.4 mg/L $K_2Cr_2O_7$

3. Test organism:

Species:	*Daphnia magna* Strauss
Age:	neonates (6 - 24 h old)
Loading:	10 mL for 5 specimens
Source:	Laboratory bred
Diet/Food:	none
Acclimation period:	Daphnids were held in groups of 25-30 organisms in 1000 mL glass vessels at test conditions. Specimens were fed on green algae and water was renewed 3 times a week.

4. Environmental conditions:

Temperature:	18 – 22°C (± 1°C during the test)
Photoperiod:	16 hours light / 8 hours dark, 600 – 700 lux
pH:	7.5 – 8.5
Dissolved oxygen:	> 60% of air saturation (approx. 6.0 mg O_2/L)
Conductivity:	0.049 µS/cm
Hardness:	14.5° dH

5. Experimental dates: January 4th, 1994 to January 6th, 1994

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The acute toxicity test was performed under static conditions as limit test using a nominal test concentration of 100 mg test item/L, corresponding to 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate/L in glass vessels containing reconstituted water (Elendt-medium). In addition, a control group was exposed to Elendt-medium. Two reference groups were equally exposed to 0.4 and 1.4 mg/L of $K_2Cr_2O_7$. There were four vessels per treatment, each containing five *Daphnia magna* (25 mL volumetric cylinder containing 10 mL test medium).

2. Observations: The *Daphnia magna* were observed 24 and 48 hours after initiation of the test. Temperature, pH-value and oxygen saturation of the test solutions were measured at initiation and test termination.

Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.

Analytical measurement of the test item concentration was performed by mean of HPLC analysis at the beginning (0 h) and end (48h) of the limit test. Glyphosate isopropylamine salt concentrations were

determined based on the concentrations of glyphosate.

3. Statistical calculations: Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The average recovery of glyphosate in the test media at the beginning (0 h) and end (48h) of the limit test were 103.7%, and 103.2% respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.4.1-16: Analytical results

	Nominal concentration [mg/L]	**Measured concentration [mg/L]**		**% of nominal**	
		24 hr	**48 hr**	**24 hr**	**48 hr**
test item	100	-	-	-	-
glyphosate isopropylamine salt	61.6	-	-	-	-
glyphosate	45.65	47.32	47.09	103.7%	103.2%

The EC_{50} values are given below based on nominal concentrations.

Table B.9.2.4.1-17: Endpoints

Endpoints	**test item [mg/L]**	**Glyphosate isopropylamine salt [mg/L]**	**Glyphosate [mg a.e./L]**
EC_{50} (48 h)	> 100	> 61.6	> 45.64

B. OBSERVATIONS

The immobility rate in the control group did not exceed 10% (0% in the test) at any stage of the test. At the concentration level of 100 mg test item/L, none of the daphnids tested were found to be immobilised, 24 h and 48 h after the start of the test.

Table B.9.2.4.1-18: Immobilisation of daphnids exposed to glyphosate isopropylamine salt

	Control	**Test item [mg/L]**	**Reference [mg/L]**	
test item	-	**100**		
glyphosate isopropylamine salt	-	**61.6**	**0.4**	**1.4**
glyphosate	-	**45.64**	**0.4**	**1.4**
24 h	0	0	0	85
48 h	0	0	5	100

The 48 h EC_{50} obtained for the reference substance was within the range of 0.4 to 1.4 mg/L, documenting the functional integrity of the test system. All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a 48-hours static acute toxicity study with *Daphnia magna* exposed to glyphosate isopropylamine salt, the EC_{50} was determined to be >100 mg test item/L, corresponding to 61.6 mg a.s/L or 45.64 mg a.e./L (nominal). As this was conducted as a limit test, the NOEC corresponds to ≥ 45.64 mg a.e./L.

All validity criteria according to the OECD 202 were fulfilled, the study is therefore considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

This study is considered valid.

48-hours static acute toxicity study with *Daphnia magna*:
EC50 > 61.6 mg glyphosate isopropylamine salt/L or 45.64 mg glyphosate acid/L (nominal).

Data point	CA 8.2.4.1/008
Report author	[redacted]
Report year	1993
Report title	Information not available
Report No	94-00549
Document No	-
Guidelines followed in study	Information mentioned in the Monograph 2001: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015).
Short description of study design and observations	Acute and chronic toxicity of glyphosate isopropylamine salt (purity 61 – 65 %) to aquatic organisms (*Daphnia magna*), 48 hours static test.
Short description of results	LC_{50} (48 h) >1000 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	The study is considered as supportive because the report is not available; so it cannot be concluded on the study validity according the current guideline requirements. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.
Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

Assessment and conclusion by RMS:

RMS notes that this study (reference 94-00549) was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

This study was requested to the BVL but could not be provided to RMS.
For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical.
The endpoint measured in this study is not critical and therefore its absence has no consequence on the risk assessment.

Data point:	CA 8.2.4.1/009
Report author	[redacted]
Report year	1990
Report title	48-Hour Acute Toxicity of Glyphosate Technical to *Daphnia magna* (OECD-Immobilization Test)
Report No	272968
Document No	-
Guidelines followed in study	OECD Guideline 202 (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004):* *Minor:* • *The pH was not in a range of 6-9, but from 2.3 – 7.6.*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid with restriction (pH issue)

Summary
The effects of glyphosate technical on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The toxicity test was performed using five nominal concentrations, 62.5, 125, 250, 500 and 1000 mg test item/L. Furthermore, a blank control consisting of reconstituted water and a stability control with 1000 mg test item/L and no daphnids were tested. Two replicates with ten daphnids each were exposed to the test item concentrations and the control. Immobilisation was recorded 24 and 48 hours after the test initiation. Dissolved oxygen and pH were recorded at the beginning and at the end of the tests.
Test item concentrations were verified in the freshly prepared and in the aged test media. During the test period of 48 hours the daphnids were exposed to a mean concentration of 86.1% of nominal concentration. Therefore, all reported results are related to nominal concentrations of the test item.

The immobilisation of *Daphnia magna* increased with increasing test concentration, while at increasing test concentrations, the pH decreases beyond the pH range of 6 - 9 given in the guideline.

The authors concluded that the EC_{50} (48 h) was 84.0 mg a.e./L with a 95% confidence interval of 73.3 to 96.6 mg a.e./L based on nominal concentrations. All validity criteria according to the guideline OECD 202 were fulfilled. The study is considered to be valid.

RMS concluded that EC50 should be considered greater than 62.5 mg/L as effects seen at other doses may be due to pH decrease of test media. The NOEC is equal to 62.5 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9%

2. Vehicle of test material and positive control: Vehicle: Test medium
Positive control: Reference item: Potassium dichromate ($K_2Cr_2O_7$)

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 20 mL test medium
Source:	In-house culture
Diet/Food:	Not stated
Acclimation period:	~ 24 h (acclimatisation started on July 2nd, test started on July 3rd).

4. Environmental conditions:

Temperature:	21.0 ± 0.5 °C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.3 – 8.2 (control) 6.3 – 7.6 (62.5 mg test item/L) 4.8 – 5.2 (125 mg test item/L) 3.2 – 3.4 (250 mg test item/L) 2.7 – 2.9 (500 mg test item/L) 2.3 – 2.6 (1000 mg test item/L)
Dissolved oxygen:	8.3 – 8.1 mg O_2/L (mean)
Conductivity:	Not stated
Hardness:	250 mg $CaC0_3$/L (reconstituted water)

5. Experimental dates: July 3rd, 1990 to July 5th, 1990

B: STUDY DESIGN AND METHODS

1. Experimental treatments: Five test concentrations (nominal 62.5, 125, 250, 500 and 1000 mg test item/L), prepared with reconstituted water according to EEC directive, were tested in duplicate.

The test was conducted in a static test setup for 48 hours in 50 mL beakers containing 20 mL test solution each. In addition, a control group was exposed to test medium without test substance or other additives. The test vessels contained 10 daphnids each. Also a stability control with 1000 mg test item/L without daphnids was tested.

2. Observations: Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after test initiation. The pH-values and oxygen saturation were measured ion each test vessel at test initiation and termination. Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis using samples taken at test start (0 h) and test termination (48 h).

3. Statistical calculations: The EC_{50} was estimated by using the Logit-model, EC_0, EC_{50} and EC_{100}

values were determined by linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed on all test concentrations and the stability control at test initiation and test termination. At 62.5, 125, 250 and 500 the test concentrations were in the range of 78.5 – 94.9% of nominal at test initiation and 77.6 – 95.2% at test termination. At the highest test concentration of 1000 mg test item/L, the concentration at test initiation was 69.7% of nominal and at test termination 85.3%, respectively. During the test period of 48 hours the daphnids were exposed to a mean concentration of 86.1% of nominal concentration. Therefore, all reported results are related to nominal concentrations of the test item.

Table B.9.2.4.1-19: Analytical results

Nominal concentration [mg test item/L]	% of nominal	
	0 hrs	48 hrs
62.5	80.9	89.1
125	78.5	77.6
250	92.4	93.4
500	94.9	95.2
1000	69.7	85.3

The EC_{50} value is given below based on nominal concentrations.

Table B.9.2.4.1-20: Endpoints

Endpoints	Glyphosate technical [mg a.e./L]
48 h EC_{50} (95% CL), Logit-model	84.0 (73.3 – 96.6)

Reference item: The 48h-EC_{50} for the reference item was 1.32 mg/L (95% CL = 1.203 – 1.426 mg/L), which was within the range of expected responses. Hence, the sensitivity of this batch of D*aphnia magna* was in agreement with the historical data collected at test facility.

B. OBSERVATIONS

The immobilisation increases with increasing test concentration. Beginning with 125 mg test item/L, all daphnids are immobilised after 48 h. At increasing test concentrations, the pH decreases beyond the pH range of 6 - 9 given in the guideline.

Table B.9.2.4.1-21: Observations of pH and immobilisation

	Control	Glyphosate [mg a.e./L]									
Test parameters	-	62.5		125		250		500		1000	
Replicate No.		1	2	1	2	1	2	1	2	1	2
% immobile daphnids after 24 h	0	10	0	30	60	100	100	100	100	100	100
% immobile daphnids after 48 h	0	10	0	100	100	100	100	100	100	100	100
pH after 24 h	8.4	6.3		4.8		3.2		2.7		2.3	
pH after 48 h	7.9	7.6		5.2		3.4		2.9		2.6	

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The EC_{50} (48 h) for *Daphnia magna* exposed to glyphosate technical was 84.0 mg a.e./L with a 95% confidence interval of 73.3 to 96.6 mg a.e./L based on nominal concentrations.

In the RAR 2015, results were recalculated based on mean measured concentrations using probit analysis which provided an EC50 value of 74 mg a.e./L (95% CL: 16.966 -130.338). The NOEC was determined to be 53 mg a.e./L.

The validity criteria according to the OECD 202 were fulfilled, the study is therefore considered valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:
This study is considered valid

The pH values at 125, 250, 500 and 1000 mg/L (pH of 4.8, 3.2, 2.7, 2.3 respectively) were below the recommended range of 6-9. This deviation was negatively correlated with increasing concentrations indicating that this pH decrease was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The high mortality observed at these concentrations can be explained by the acidity of the test solution.

The endpoints proposed by the study authors are based on nominal concentrations. However, measured concentration was below the range of 80-120% of the nominal of 125 mg/L. Then the endpoints should be based on mean measured concentrations. In the RAR 2015, results were recalculated based on mean measured concentrations using probit analysis which provided an EC50 value of 74 mg a.e./L (95% CL: 16.966 -130.338). The NOEC was determined to be 53 mg a.e./L. RMS agrees with the recalculated endpoints.

However, effects observed at concentrations of 125 mg/L and above can not be taken into account in EC50 calulation because effects may be due to pH decrease. pH are within acceptable range up and including 62.5 mg a.e./L. Endpoints are expressed as nominal concentrations as the measured concentrtaions was found to be 80.9% at 24h and 89.1% at 48h.
.
EC50 > 62.5 mg glyphosate acid/L (nominal)
NOEC = 62.5 mg glyphosate acid/L

Data point:	CA 8.2.4.1/010
Report author	[redacted]
Report year	1981
Report title	Acute Toxicity of MON 0139 (Lot LURT 12011) (AB-81-074) to *Daphnia magna*
Report No	27203
Document No	-
Guidelines followed in study	Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, US EPA, Ecol Res. Ser. 660/3-75009
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the current guideline OECD 202 (2004):* *Major:* *- No analytical measurements of the lowest and highest treatment solutions were performed.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary
The effects of MON 0139 on *Daphnia magna* were evaluated in a 48-hour static toxicity test. The test was performed using six nominal concentrations of 56, 100, 180, 320, 560 and 1000 mg test item/L in duplicates and included a blank control group (daphnia medium only). Twenty daphnids (2 replicates, 10 individuals per replicate) were exposed to each treatment level and in the control group. Total number of immobile *Daphnia magna* in each vessel were recorded at 24 and 48 hours after the test initiation. The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination. In addition, total hardness and specific conductivity of the dilution water was analysed.
The 48 h LC_{50} for *Daphnia magna* exposed to MON 0139 was determined to be 930 mg test item/L. The no effect level (NOEC) observed for MON 0139 was 320 mg test item/L after 48 hours. According to the points deviated from the current guideline OECD 202 recommendations, the study is considered as supportive.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 0139
Description:	Light yellow liquid
Lot/Batch #:	LURT 12011
Purity:	62.49%

2. Vehicle of test material/media: water

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (1st instar, < 24 h old)
Loading:	10 specimens in 200 mL test solution

Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

4. Environmental conditions:

Temperature:	20 ± 1°C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.6 (control, test start), 7.9 – 8.6 (control and test item at test end)
Dissolved oxygen:	8.8 mg/L (control, test start), 3.5 – 7.8 mg/L (control and test item at test end)
Conductivity:	50 µmhoS/cm
Hardness:	255 ppm ($CaCO_3$).

5. Experimental dates: April 21 to April 24 1981

B. STUDY DESIGN

Experimental treatments

The toxicity of MON 0139 on *Daphnia magna* was evaluated in a 48-hour static toxicity test, using nominal concentrations of 56, 100, 180, 320, 560 and 1000 mg test item/L. In addition, a control group was exposed to dilution water. The test solutions were prepared using water prepared to a total hardness of 255 mg $CaCO_3$/L. There were two glass jars per treatment, each containing ten daphnids (250 mL glass jars containing 200 mL test medium). The vessels were kept at 20 ± 1°C. The photoperiod was controlled to give 16 hours daylight and 8 hours darkness.

Observations

Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation. The pH-values and oxygen saturation of the test solutions were measured at test initiation (only in control) and at test termination (control and three test concentrations). In addition, total hardness and specific conductivity of the dilution water was analysed.

The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 per cent of the daphnids should have been immobilised or show or other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

Statistical calculations

The LC_{50} values were obtained by employing a computerised LC_{50} program developed by Stephan et. al. (1978) performing binomial, moving average and probit tests.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 48 hours LC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.4.1-22: Endpoints

Endpoints	MON 0139 [mg/L]	Glyphosate [mg a.s./L]
48 hours LC_{50} (95% C.I.)	930 (800 - 1200)	581 (500 - 750)
48 hours NOEC	320	200

C.I. = Confidence interval

B. OBSERVATIONS

No mortality to *Daphnia magna* from exposure to MON 0139 was observed at test concentrations ≤ 560 mg test item/L. At 1000 mg test item/L, some behavioural effects were notified after 48 hours and 10% and 60% mortality was observed after 24 and 48 hours, respectively (see table below).

Table B.9.2.4.1-23: Mortality of *Daphnia magna* exposed to MON 0139

Test concentration (mg MON 0139/L)	Mortality (%)	
	24 hours	48 hours
Control	0	0
56	0	0
100	0	0
180	0	0
320	0	0
560	0	5
1000	10	60

The following points deviated from the current guideline OECD 202 recommendations:
- No analytical measurements of the lowest and highest treatment solutions were performed.
- The hardness is slightly higher than 250 mg/L CaCO3 (actual value: 255 mg/L)

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 48 h LC_{50} for *Daphnia magna* exposed to MON 0139 was determined to be 930 mg test item/L equivalent to 581 mg a.e./L. The no effect level (NOEC) observed for MON 0139 was 320 mg test item/L after 48 hours, equivalent to 200 mg a.e./L.

No chemical analysis was performed to confirm glyphosate concentration in the test media. The test would therefore be considered as supportive for risk assessment purposes.

Assessment and conclusion by RMS:
RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

The 48 h LC50 for *Daphnia magna* exposed to MON 0139 was determined to be 930 mg test item/L equivalent to 581 mg glyphosate acid/L (based on nominal concentrations).
The no effect level (NOEC) observed for MON 0139 was 320 mg test item/L after 48 hours, equivalent to 200 mg a.e./L.

As noted by the applicant, no chemical analysis was performed to confirm glyphosate concentration

in the test media. This study is only considered as supportive.

Data point:	CA 8.2.4.1/011
Report author	[redacted]
Report year	1978
Report title	Acute Toxicity of Technical Glyphosate (AB-78-201) to *Daphnia magna*
Report No	AB 78-201
Document No	-
Guidelines followed in study	Committee on methods for toxicity tests with aquatic organisms.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 202 (2004):* *Major:* • *no analytical verification of test concentrations*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	No, not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed)
Acceptability/Reliability (RMS):	Not reliable

Summary

The effects of glyphosate on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Based on the results of a range finding test, a definite toxicity test was performed using nominal concentrations of 560, 650, 750, 870 and 1000 mg test item/L, equivalent to 464.8, 539.5, 622.5, 722.1, and 830.0 mg glyphosate/L. In addition, a control group was exposed to dilution water. There were three vessels per treatment, each containing ten daphnids.

The total number of immobile *Daphnia magna* was recorded at 24 h and 48 h after test initiation.

At and above nominal concentrations of 870 mg test item/L, 100% immobilisation was observed, while no immobilisation was observed at a nominal concentration of 560 mg test item/L, 48 hours after the test initiation. The 48 h EC_{50} for *Daphnia magna* exposed to glyphosate was calculated to be 780 mg test item/L. The 48- hour no-effect level (NOEC) was determined to be 560 mg/L. All validity criteria according to the guideline OECD 202 were fulfilled, however no analytical verification of test concentrations was made and the study was not conducted to GLP. This study is therefore considered supportive by the applicant. RMS considers this study as not reliable as no analytic verification was performed pH values were not available.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Technical Glyphosate

Description: White powder

Lot/Batch #:	XHI-162
Purity:	83.0%
2. Vehicle of test material/media :	Well water
3. Test organism:	
Species:	*Daphnia magna*
Age:	Neonates (< 18 h old)
Loading:	10 specimens for 250 mL test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None
4. Environmental conditions:	
Temperature:	19 ± 1°C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.0 (at test termination)
Dissolved oxygen:	7.5 mg/L
Conductivity:	Not stated
Hardness:	> 250 mg $CaCO_3$/L.
5. Experimental dates:	August 29th, 1978 to August 31st, 1978

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, definite toxicity test was performed using nominal concentrations of 560, 650, 750, 870, 1000 mg test item/L, equivalent to 464.8, 539.5, 622.5, 722.1, and 830.0 mg glyphosate/L in a static test setup. The test solutions were prepared using well water of the test facility (Dissolved oxygen = 8.6 mg/L, pH = 7.8, hardness > 250 mg $CaCO_3$/L.). In addition, a control group was exposed to dilution water. There were three replicates per treatment, each containing ten daphnids (500 mL glass beakers containing each 250 mL test medium).

2. Observations: Total number of immobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation. Temperature, pH-value and oxygen saturation of the test solutions were measured at the test termination. Hardness of the test water was measured at test initiation.

3. Statistical calculations: EC_{50} values were calculated along with the 95% confidence limits using Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

No analytical verification reported.

The EC_{50} and NOEC values are given below based on nominal concentrations as no analytical verification of test concentrations was made.

Table B.9.2.4.1-24: Endpoints

Endpoints (48 h)	Test item[mg/L]	Glyphosate [mg a.e./L]
EC_{50} (95% C.I.)	780 (696 - 874)	647.4 (577.7 - 725.4)
NOEC	560	464.8

B. OBSERVATIONS

At and above nominal concentrations of 870 mg test item/L, 100% immobilisation was observed while no immobilisation was observed at the nominal concentration of 560 mg test item/L, 48 hours after the test initiation. At concentrations of 650 and 750 mg test item/L, immobilisation of 3.3% and 33.3% of specimens was observed.

Table B.9.2.4.1-25: Lethal effects of glyphosate to *Daphnia magna*

Test item [mg/L]	**Control**	**560**	**650**	**750**	**870**	**1000**
Glyphosate [mg a.e./L]	**-**	**464.8**	**539.5**	**622.5**	**722.1**	**830.0**
Immobility (24 h) [%]	0	0	0	6.7	73.3	100
Immobility (48 h) [%]	0	0	3.3	33.3	100	100

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The 48 h EC_{50} for *Daphnia magna* exposed to technical glyphosate was calculated to be 780 mg test item/L, equivalent to 647.4 mg a.e./L. The 48- hour no-effect level (NOEC) was determined to be 560 mg/L, equivalent to 464.8 mg a.e./L.

All validity criteria according to the guideline OECD 202 were fulfilled, however no analytical verification of test concentrations was made. This study is therefore considered supportive for risk assessment purposes.

Assessment and conclusion by RMS:
This study was not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed).
No analytic verification was performed and therefore dose verification is impossible.
The pH values were not available. Only a single value (pH 8.0) was reported in the raw data. On the basis of the current knowledge, it is obvious that such concentrations (of technical glyphosate) would have decreased the pH in the test media. The (low) effect observed in this study are likely to be underestimated.

RMS considers this study as not reliable.

Data point:	CA 8.2.4.1/012
Report author	[redacted]
Report year	1998
Report title	Acute Toxicity Study in *Daphnia magna* with (Aminomethyl)Phosphonic Acid (Static)
Report No	232471
Document No	-
Guidelines followed in study	OECD Guideline 202, Part I (1984) ECC Directive 92/69, Part C.2 (1992) ISO International Standard 6341 (1996)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004): none*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Summary

The effects of (Aminomethyl) phosphonic acid (AMPA) on *Daphnia magna* were evaluated in a 48-hour static toxicity test conducted as a limit test with a nominal concentration of 100 mg test item/L. Furthermore, a blank control was tested. Twenty daphnids (2 replicates, 10 individuals per replicate) were exposed to each treatment level.

Immobilisation was recorded 24 and 48 hours after the start of the test.

At the tested nominal concentration of 100 mg test item/L, no immobilisation was observed in tested daphnids during the 48 h exposure time. The 48-h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be > 100 mg test item/L All validity criteria according to OECD 202 were fulfilled. The study is considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	(Aminomethyl)phosphonic acid
Description:	White powder
Lot/Batch #:	A010047101
Purity:	99%

2. positive control: Reference item: $K_2Cr_2O_7$

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 80 mL of test medium
Source:	In-house culture

4. Environmental conditions:

Temperature:	20.4 – 20.6°C
Photoperiod:	16 hours light / 8 hours dark
pH:	8.0 – 8.2 (control), 6.2 - 6.4 (test solution)
Dissolved oxygen:	8.8 – 9.0 mg O_2/L
Hardness:	250 mg $CaC0_3$/L
5. Experimental dates:	May 18th, 1998 to May 27th, 1998

B: STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, the final toxicity test was performed using a unique nominal concentration of 100 mg test item/L prepared using ISO-medium (in milli-RO water). The test was conducted in a static test setup as limit test. In addition, a control group was exposed to the test medium without test substance or other additives. The test consisted of two replicates per treatment group (100 mL vessels containing 80 mL test solution each). Per replicate 10 daphnids were exposed.

2. Observations: Total number of mobile *Daphnia magna* was recorded at 24 h and 48 h after the test initiation.

The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination. The temperature was controlled daily in one control vessel, starting from the beginning of the test.

Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis using samples taken at test start (0 h) and test termination (48 h).

3. Statistical calculations: Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value is given below based on nominal concentrations.

Table B.9.2.4.1-26: Endpoints

Endpoints	(Aminomethyl) phosphonic acid [mg/L]
EC_{50} (48 h)	> 100

Analytical data: Before introduction of the daphnids 98% of (Aminomethyl)phosphonic acid was recovered. In the aged test media 95% of the nominal concentration was recovered. The results are summarised below.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.4.1-27: Analytical results

Time [hours]	Nominal [mg/L]	Analysed [mg/L]	% of nominal [mg/L]
0	100	98.2	98
48	100	95.4	95

Reference test: The 48h-EC_{50} for the reference item was 0.5 mg/L (95% CL = 0.4 – 0.6 mg/L), which was within the range of expected responses. Hence, the sensitivity of this batch of D*aphnia magna* was in agreement with the historical data collected at test facility.

B. OBSERVATIONS

At the tested nominal concentration, no immobilisation was observed in tested daphnids during the 48 h exposure time. Also, all validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Under the conditions of the present test (Aminomethyl) phosphonic acid induced no visible effects in *Daphnia magna* at nominal concentrations of 100 mg/L. Hence, the 48-h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be > 100 mg/L, the maximum nominal concentration tested, and the NOEC ≥ 100 mg/L.

The study is considered valid.

Assessment and conclusion by RMS:
This study is considered valid.

48 h EC50 for *Daphnia magna* exposed to aminomethylphosphonic acid (AMPA) > 100 mg/L (nominal).
NOEC = 100 mg/L

Data point:	CA 8.2.4.1/013
Report author	[redacted]
Report year	1994
Report title	AMPA: Acute toxicity to *Daphnia magna*
Report No	X582/C
Document No	-
Guidelines followed in study	OECD No 202
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004): none*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary

The effects of AMPA on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 18, 32, 56, 100 and 180 mg/L of AMPA. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control).
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. The concentration of AMPA in the test solutions were measured at 0 and 48 hours. The mean measured test concentrations of AMPA ranged from 93 to 128% of the nominal values, therefore, the results reported are related to nominal concentrations of the test item. The 48-h EC_{50} for *Daphnia magna* exposed to AMPA was >180 mg/L. The NOEC after 48 h based on immobilisation was 180 mg AMPA/L. All validity criteria according to OECD 202 were fulfilled. The study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA technical (metabolite of glyphosate))
Description:	White solid
Lot/Batch #:	Not mentioned in the report
Purity:	85%

2. Vehicle of test material/media: Dilution water

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Less than 24 hours

Loading:	5 organisms per vessel (250 mL glass beakers containing 200 mL test solution) which corresponds to 25 *Daphnia*/L.
Source:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	19.9-20.1°C
pH:	8.23-8.47
Dissolved oxygen:	8.8-9.1 mg O_2/L
Conductivity:	545 mg/L µS/cm
Hardness:	161.6 mg $CaCO_3$
Photoperiod:	16 hours light / 8 hours dark with 15 minute transition periods

5. Experimental dates: November 16th, 1993 to November 18th, 1993

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of AMPA on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to nominal 18, 32, 56, 100 and 180 mg/L of AMPA. In addition, 4 x 5 *Daphnia* were exposed to test medium without test substance (blank control). A stock solution of nominal concentration of 180 mg a.s./L was prepared by dissolving 0.36 mg test item in 2 L dilution water. This stock solution was observed to be clear and colourless. One litre of each test solution was prepared by the addition of aliquots of stock solution to dilution water. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations: Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. The concentrations of AMPA in the test solutions were measured at 0 and 48 hours.

3. Statistical calculations: The EC_{50} could not be quantified due to the absence of toxicity of the test item, therefore, no statistical analysis was performed.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured test concentrations of AMPA ranged from 93 to 128% of the nominal values. The limit of quantification of AMPA in this study was 6.9 mg/L. The results are summarised below. The variability of the chemical analysis was considered to be due to the analytical method used. A similar study with AMPA technical completed after this study with an improved analytical method, reported mean measured concentrations ranging from 100 to 111% of the nominal values. Therefore, it was assumed that the nominal concentrations were maintained during this study and results have been provided using the nominal concentrations.

Table B.9.2.4.1-28: Analytical results

Nominal concentration [mg AMPA/L]	Measured concentration [mg AMPA/L]		Mean measured concentration	
	0 hrs	48 hrs	[mg AMPA/L]	% of nominal
Control	<6.9	<6.9	<6.9	-
18	34	11	23	128
32	45	30	38	119
56	73	47	60	107
100	99	86	93	93
180	170	200	190	106

The 24 and 48 hour EC_{50} values (based on nominal concentrations of AMPA) are given below.

Table B.9.2.4.1-29: EC_{50} values for *Daphnia magna*

Time	EC_{50} (mg a.s./L)	95 % confidence interval (mg a.s./L)
24 h	>180	-
48 h	>180	-

B. OBSERVATIONS

The effects of AMPA on *Daphnia magna* are shown below.

Table B.9.2.4.1-30: Effects of AMPA on *Daphnia magna* exposed for 48 hours

Nominal concentration [mg a.s./L]	Number of exposed *Daphnia* per replicate	Number of immobile *Daphnia* after 24 hours	Immobility after 24 hours [%]	Number of immobile *Daphnia* after 48 hours	Immobility after 48 hours [%]
Control	20	0	0	0	0
18	20	0	0	0	0
32	20	0	0	0	0
56	20	0	0	0	0
100	20	0	0	1	5
180	20	0	0	0	0

All validity criteria according to OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48-h EC_{50} for *Daphnia magna* exposed to AMPA was >180 mg/L based on nominal concentration. The NOEC after 48 h based on immobilisation was ≥ 180 mg/L.

All validity criteria according to OECD 202 were fulfilled, so the study is therefore considered valid.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study is considered valid.
48-h EC50 for *Daphnia magna* exposed to AMPA >180 mg/L (based on nominal concentration).
NOEC =180 mg/L

Data point:	CA 8.2.4.1/014
Report author	[redacted]
Report year	1991
Report title	Acute Toxicity of AMPA to *Daphnia magna*.
Report No	38988
Document No	-
Guidelines followed in study	Guideline No. 72-2, U.S. EPA-FIFRA 40 CFR. Part 158, 145
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from to the guideline OECD 202 (2004): none*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Supportive

Executive Summary

The effects of AMPA on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Based on the results of a range finding test, the final toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L prepared using hard blended water (a combination of well water and reverse-osmosis water blended to a hardness of 160-180 mg/L as $CaC0_3$) Furthermore, a control group was exposed to the dilution water (hard blended water). The test consisted of two replicates per treatment group. Per replicate 10 daphnids were exposed.

Total number of immobile *Daphnia magna* was recorded at 3, 24 h and 48 h after the test initiation. In addition, other abnormal effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of the test chambers were recorded.

At the highest test concentration (1000 mg test item/L), 85% and 100% immobility were observed at 24 and 48 hours after test initiation. At or below a concentration of 320 mg test item/L, no mortality was observed.

Immobility and abnormal effects, namely surfacing and daphnids trailing extraneous material were observed in the 560 and 1000 mg/L test concentrations. The abnormal effects such as daphnia on the bottom of the test vessel and immobility at 24- and 48- hours, respectively, in the control were considered aberrant since no toxic response was observed at 100, 180 and 320 mg/L test concentrations. The 48 h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be 690 mg AMPA/L (nominal). The 48- hour no-effect level (NOEC) was determined to be 320 mg/L (nominal). All validity criteria according to the OECD guideline 202 were fulfilled. The study is therefore considered valid by the applicant.

RMS considered this study as informative only (analytical results not available). No validation data for analytical method was available (see Volume 3 CA B.5). This is not a validity criteria, thus the study is still supportive but the overall reliability (and weight in weight of evidence) is reduced.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA
Description:	White powder
Lot/Batch #:	HET-9001-1463T
Purity:	94.38%

2. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 200 mL of test medium
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None

3. Environmental conditions:

Temperature:	20 ± 1°C
Photoperiod:	16 hours light / 8 hours dark (399-797 Lux), with 30 minute dawn dusk transition periods.
pH:	8.2 – 8.3 (control), 5.2 (highest test concentration)
Dissolved oxygen:	8.4 – 8.8 mg O_2/L (94% - 101% of O_2 saturation)
Conductivity:	370 µS/cm
Hardness:	160 mg $CaC0_3$/L

4. Experimental dates: November 24, 1990 to November 26, 1990

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, the final toxicity test was performed using nominal concentrations of 100, 180, 320, 560 and 1000 mg test item/L dissolved in hard blended water (a combination of well water and reverse-osmosis water blended to a hardness of 160 mg/L as $CaC0_3$). The test was conducted in a static test setup. In addition, a control group was

exposed to dilution water (hard blended water). The test consisted of two replicates per treatment group in 250 mL glass beakers containing 200 mL test solution. 10 daphnids were exposed per replicate.

2. Observations: Total number of immobile *Daphnia magna* was recorded 3, 24 h and 48 h after test initiation. In addition, other effects such as surfacing, clumping of the daphnids together and daphnids tending to the bottom of the test chambers were recorded.
The pH-values and oxygen saturation of the test solutions were measured at test initiation and termination (0 – 48 h). The temperature was recorded continuously in all test vessels, starting from the test initiation.

Analytical samples of the control water and each test level solutions were taken at the beginning and the end of exposure. These samples were frozen and sent to the study sponsor at test termination. The results of these analyses are reported separately Monsanto (Study No. ML-90-403/EHL-90187-Daphnia)

3. Statistical calculations: The EC_{50} values were determined by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical results
The results of analytical part are reported in a separate study (Monsanto study No. ML-90-403/EHL-90187-Daphnia).

The EC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.4.1-31: Endpoints

Endpoints	AMPA [mg/L]
EC_{50} (48 h) (95% CI)	690 (560 – 1000)
NOEC (48 h)	320

B. OBSERVATIONS

At highest test concentration (1000 mg test item/L), 85% and 100% immobility were observed at 24 and 48 hours after test initiation. At or below a concentration of 320 mg test item/L, no mortality was observed.
Immobility and abnormal effects such as surfacing and daphnids trailing extraneous material were observed in the 560 and 1000 mg/L test concentrations. The abnormal effects such as daphnia on the bottom of the test vessel and immobility at 24- and 48- hours, respectively, in the control were considered aberrant since no toxic response was observed at 100, 180 and 320 mg/L test concentrations.

Table B.9.2.4.1-32: Lethal and sublethal effects of AMPA to *Daphnia magna*

		Control	AMPA [mg/L]				
			100	180	320	560	1000
24 h	Cumulated Immobility [%]	5	0	0	0	0	85
	Symptoms	5% OB	-	-	-	5% OB	-
48 h	Cumulated Immobility [%]	0	0	0	0	15%	100
	Symptoms	-	-	-	-	5% SUR/TR	-

SUR = surfacing; OB = on bottom of test vessel; TR = trailing extraneous material

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 h EC_{50} for *Daphnia magna* exposed to AMPA was determined to be 690 mg/L (nominal). The 48- hour no-effect level (NOEC) was determined to be 320 mg/L (nominal).

All validity criteria according to the OECD 202 were fulfilled. The study is therefore considered valid and reliable for risk assessment purposes.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

Immobility was observed at the two highest concentrations (560 and 1000 mg/L). The pH values at these concentrations (5.6 and 5.2 respectively) were below the recommended range (recommended pH 6-9). This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of test item). The high mortality observed at these concentrations can be explained by the acidity of the test solutions (at least partly).

According to the study report, analytical control measurements of the actual concentrations of the test item were performed and the results are reported in a separate study (study number: ML-90-403/EHL-90187-Daphnia). The study ML-90-403 was checked by RMS however analytical results were not found (table of results was missing). For this reason, RMS considers the results of this study (38988) as informative only. No validation data for analytical method was available (see Volume 3 CA B.5). This is not a validity criteria, thus the study is still supportive but the overall reliability (and weight in weight of evidence) is reduced.The reliability of this study might be revised once analytical results are made available.
This study is considered as informative only (analytical results not available).

48 h EC50 for *Daphnia magna* exposed to AMPA = 690 mg/L (nominal).
NOEC= 320 mg/L (nominal).

Data point:	CA 8.2.4.1/015
Report author	[redacted]
Report year	2011
Report title	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*)
Report No	139A-395
Document No	-
Guidelines followed in study	OECD 202 (1984) EPA OPPTS 850.1010
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 202 (2004): none*
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary

The toxicity of Hydroxymethylphosphonic acid (HMPA) on *Daphnia magna* was evaluated in a 48-hour static toxicity test. *Daphnia magna* neonates were exposed to a limit concentration of 100 mg HMPA/L and a negative control consisting of dilution water only. The test consisted of three replicates per treatment group and control with 10 daphnids exposed per replicate vessel. *Daphnia* were not fed during the test. All Daphnids were observed for immobilisation and other clinical signs of toxicity at 2.5, 24 and 48 hours after test initiation.

Temperature, pH-values and dissolved oxygen concentrations were measured at the beginning, at approximately 24 hours during the test and at the end of the test. Samples of the control and the test item treatment media were taken and analysed for HMPA concentration at the beginning of the test and at 48 hours from each replicate test chamber. HMPA was not detected in the control group. The measured test concentrations ranged between 86 and 103% of the nominal values.

There was no immobility or overt signs of toxicity observed in the treatment group or in the control. The 48-hour EC_{50} for *Daphnia magna* exposed to HMPA was > 100 mg HMPA/L. The 48- hour NOEC was determined to be ≥ 100 mg HMPA/L. All validity criteria according to the OECD guideline 202 were fulfilled. The study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: HMPA(Hydroxymethylphosphonic acid)

Description: White powder

Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0%
2. Vehicle of test material/media:	Vehicle: Well water
3. Test organism:	
Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	10 daphnids per 220 mL of test medium
Source:	In-house culture
Diet/Food:	None
Acclimation period:	None
4. Environmental conditions:	
Temperature:	19.7 – 20.7 °C
Photoperiod:	16 hours light (light intensity = 323 Lux), with 30 minute transition periods.
pH:	6.9 – 8.5
Dissolved oxygen:	8.3 – 9.4 mg O_2/L (≥92% of O_2 saturation)
Conductivity:	386 µS/cm
Hardness:	140 mg $CaC0_3$/L
5. Experimental dates:	January 25, 2011 to January 28, 2011

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity of Hydroxymethylphosphonic acid (HMPA) on neonates of *Daphnia magna* was evaluated in a 48-hour static toxicity test at a single nominal limit concentration of 100 mg HMPA/L dissolved in well water. A negative control group (well water only) was prepared in parallel. Thirty daphnids (3 replicates of 10 animals per test beaker) were exposed at the control and at the limit concentration.

2. Observations: The total number of immobile *Daphnia magna* was recorded at 2.5, 24 h and 48 h after test initiation. In addition, specimens were observed for clinical signs of toxicity.

Temperature, pH-values and oxygen saturation of the test solutions were measured at test initiation, after 24 hours and at test termination (48 h). The temperature of test media was monitored continuously in all test vessels. Hardness, alkalinity, specific conductance and total organic carbon (TOC) were measured at the beginning of the test.

Samples of test media were taken from each replicate test chamber at the start and end of the test for the determination of HMPA concentrations. Samples were analysed using an HPLC method of analysis with mass selective detection (LC/MS).

The validity criteria according to the current OECD 202 guideline are the following:

- In the control, not more than 10 per cent of the daphnids should have been immobilised or show or other signs of disease or stress.
- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels.

3. Statistical calculations: Descriptive only since no immobility of daphnids was observed in the test and control treatments.

II. RESULTS AND DISCUSSION

A. FINDINGS

The measured test concentrations ranged between 85.9 and 103% of the nominal values.

Table B.9.2.4.1-33: Analytical results

Nominal HMPA [mg/L]	0 mg/L	100 mg/L
0 h	< LOQ*	85.9
48 h	< LOQ*	95.8
	< LOQ*	99.6
	< LOQ*	103.0
Mean measured HMPA [mg/L]	-	93
% of nominal	-	93

*LOQ = 1.00 mg/L

Therefore, the EC_{50} and NOEC values given below are based on nominal concentrations.

Table B.9.2.4.1-34: Endpoints

Endpoints	HMPA [mg/L]
48 h EC_{50}	>100 mg/L (nominal)
48 h NOEC	≥ 100 mg/L (nominal)

B. OBSERVATIONS

After 2.5, 24 and 48 hours of exposure, no immobilisation of *Daphnia* in the control nor in the test item concentration vessels was observed.

Table B.9.2.4.1-35: Acute toxicity of MON 52276 to *Daphnia magna* under flow-through conditions

Nominal concentration HMPA (mg a.s./L)	Time point (h)	Abnormalities/ Sublethal Effects	No. of *Daphnia* immobilised or dead[1]	Cumulative % mortality
0	2.5 24 48	None observed	0 0	0 0
100	2.5 24 48	None observed	0 0	0 0

[1] Of 30 total *Daphnia* in group.

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48-hour EC_{50} for *Daphnia magna* exposed to HMPA was >100 mg/L (nominal). The 48- hour NOEC was determined to be ≥ 100 mg/L (nominal).

All validity criteria according to the OECD 202 were fulfilled. The study is therefore considered valid and reliable for the regulatory risk assessment of glyphosate.

Assessment and conclusion by RMS:

This study is considered valid.
48-hour EC50 for *Daphnia magna* exposed to HMPA > 100 mg a.s./L nominal (>93 mg/L measured).
NOEC = 100 mg HMPA/L (>93 mg/L measured).

B.9.2.4.2. Acute toxicity to an additional aquatic invertebrate species

Data point:	CA 8.2.4.2/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to mysid shrimp (*Mysidopsis bahia*)
Report No	AB0503/H
Document No	-
Guidelines followed in study	EPA FIFRA, Subdivision E, Guideline 72-3
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviation from the guideline OCSPP 850.1035 (2016): none*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid with restriction (pH issue)

Executive Summary

The effects of glyphosate acid on mysid shrimp *Mysidopsis bahia* were evaluated in a 96-hour static toxicity test. Ten mysids were allocated to a single vessel (1000 mL glass beaker containing 800 mL test solution) for each test concentration and the dilution water control. The shrimps were exposed to nominal 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg a.s./L, together with pH adjusted 320, 560, and 1000 mg a.s./L.

The mysids were exposed to the test item for 96 hours at 25±1°C. The mysids were fed on days 0, 1, and 3 with *Artemia salina* nauplii.

Mortalities of the mysids and overt symptoms of toxicity were assessed after 24, 48, 72, and 96 hours.

pH-values were determined in the test media at the beginning and at the end of the test. Dissolved oxygen concentrations were measured at 0, 48, and 96 hours. The water temperature in the test vessels was measured daily. The salinity of the dilution water control and 1000 mg/L solution was determined at the start and at the end of the test. The concentrations of glyphosate acid in the test solutions were measured at 0, 48, and 96 hours.

At the lowest test concentration of 3.2 mg/L, analytical results indicated that an error might have occurred during the solution preparation, leading to a value 150% of nominal. Since this was a no effect concentration, and several higher concentrations gave no indication of toxicity, this data point was excluded from all calculations. Excluding this concentration, the mean measured concentrations ranged from 81 to 95% of the nominal values. On the basis of the analytical data the nominal concentrations were used for the calculation and reporting of all results.

The authors concluded that the 96-h LC_{50} for *Mysidopsis bahia* exposed to glyphosate acid was 80 mg/L based on nominal concentration. The NOEC after 96 h was 32 mg test item/L. In test systems dosed with pH adjusted glyphosate acid, no mortalities at a nominal concentration of 560 mg a.s./L and 50% mortality at 1000 mg a.s./L indicated this 96-h LC_{50} (80 mg/L) was caused by the low pH of the unneutralised glyphosate acid solutions.

The validity criteria of OCSPP 850.1035 were fulfilled so the study is therefore considered valid.

Given that the proposed 96 hour LC50 for *Mysidopsis bahia* exposed to glyphosate acid of 80 mg a.s./L and its 95% confidence interval based on nominal concentrations is between 56 and 100 mg/L, RMS considered the LC50 suitable for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%

2. Vehicle of test material/media and positive control: Vehicle: Dilution water (1:1 mix of dechlorinated tap water and full seawater

Positive control: Not stated

3. Test organism:

Species:	Mysid shrimp *Mysidopsis bahia*
Source of organisms:	Continuous cultures at Brixham Environmental Laboratory
Age of animals:	Less than 24 hours
Loading:	0.8 mysids per litre of water

4. Environmental conditions:

Temperature:	23.7-25.9°C
pH:	4.5-8.0 (unneutralised test solutions) 8.0-8.5 (neutralised test solutions)
Dissolved oxygen:	7.0-8.4 mg O_2/L
Salinity:	17%
Photoperiod:	16 hours light / 8 hours dark with 20 minute transition periods

5. Experimental dates: March 21, 1996 to March 25, 1996

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate acid on mysid shrimp *Mysidopsis bahia* were evaluated in a 96-hour static toxicity test. Ten mysids were allocated to a single vessel (1000 mL glass beaker containing 800 mL test solution) for each test concentration and the dilution water control. The shrimps were exposed to nominal 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg a.s./L, together with pH adjusted 320, 560, and 1000 mg a.s./L.

A stock solution of nominal concentration of 1000 mg a.s./L was prepared by dispersing 1.5 g test item in 1.5 L of dilution water. The unneutralised test solutions were prepared by dispersing aliquots of the stock solution to dilution water. Three further test solutions were prepared at 320, 560, and 1000 mg a.s./L from a stock solution of 1000 mg/L prepared by dispersing 2.0 g of glyphosate acid in approximately 2 L of dilution water and adjusted to pH 8.1 with 1M sodium hydroxide.

The mysids were randomly placed into the test beaker and exposed to the test item for 96 hours at 25±1°C. The mysids were fed on days 0, 1, and 3 with *Artemia salina* nauplii.

2. Observations: Mortalities of the mysids and overt symptoms of toxicity were assessed after 24, 48, 72, and 96 hours. pH-values were determined in the test media at the beginning and at the end of the test. Dissolved oxygen concentrations were measured at 0, 48, and 96 hours. Treatments showing 100% mortality were measured for pH and dissolved oxygen at that time. The water temperature in the test vessels was measured daily. The salinity of the dilution water control and 1000 mg/L solution was determined at the start and at the end of the test. The concentrations of glyphosate acid in the test solutions were measured at 0, 48, and 96 hours.

3. Statistical calculations: The LC_{50} values were calculated by the Brixham Environmental Laboratory computer program "LC_{50}" using Stephan's method.

II. RESULTS AND DISCUSSION

A. FINDINGS

At the lowest test concentration of 3.2 mg/L, analytical results indicated that an error might have occurred during the preparation of the test solution, leading to a value 150% of nominal. Since this was a no effect concentration, and several higher concentrations gave no indication of toxicity, this data point was excluded from all calculations. Excluding this concentration, the mean measured concentrations ranged from 81 to 95% of the nominal values. Based on the analytical data the nominal concentrations were used for the calculation and reporting of all results.

Table B.9.2.4.2-1: Analytical results

Nominal concentration of Glyphosate acid [mg/L]	Measured concentration of glyphosate acid [mg/L]			Mean measured concentration of glyphosate acid [mg/L]	% of nominal
	0 h	48 h	96 h		
Dilution water control	< 0.01	< 0.01	< 0.01	< 0.01	-
3.2	4.8	4.1	5.5	4.8	150
5.6	4.7*	4.1*	5.5*	4.8	86
10	7.9	7.0	9.5	8.1	81
18	16	15	16	16	89
32	30	28	30	29	91
56	55	48	50	51	91
100	98	89	97	95	95
180	170	160	-	170	94
320 (pH adjusted)	300	270	290	290	91
560 (pH adjusted)	530	490	550	520	93
1000 (pH adjusted)	940	860	970	920	92

*mean of triplicate analysis. The LOQ was 0.01 mg glyphosate acid/L.

The LC_{50} values for *Mysidopsis bahia* (based on nominal concentrations of glyphosate acid) are given below.

Table B.9.2.4.2-2: Endpoints

Time	LC_{50} [mg a.s./L]	95 % confidence interval [mg a.s./L]
24 h	130	100-180
48 h	96	77-130
72 h	88	71-110
96 h	80	64-100

The 96-hour NOEC was 32 mg a.s./L.

B. OBSERVATIONS

The effects of glyphosate acid on *Mysidopsis bahia* are shown in the table below.

Table B.9.2.4.2-3: Effects of glyphosate acid on *Mysidopsis bahia*

Nominal concentration (mg a.s./L)	Cumulative percentage mortality observed			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
3.2	0	0	0	0
5.6	0	0	0	0
10	0	0	0	0
18	0	0	0	0
32	0	0	0	0
56	0	0	0	10
100	0	60	80	80
180	100	100	100	100
320 (pH adjusted)	10	10	10	10
560 (pH adjusted)	0	0	0	0
1000 (pH adjusted)	0	0	30	50

In test systems dosed with pH adjusted glyphosate acid, no mortalities at a nominal concentration of 560 mg a.s./L and 50% mortality at 1000 mg a.s./L indicated this 96-h LC_{50} (80 mg/L) was caused by the low pH of the unneutralised glyphosate acid solutions.

The validity criteria of OCSPP 850.1035 Mysid Acute Toxicity Test (October 2016) were fulfilled as:

- All test vessels were identical
- Individual test organisms were randomly assigned to test vessels.
- A dilution water control was included in the test
- Not more than 10% of the organisms in the dilution water control showed signs of disease, stress (*e.g.*, discoloration, unusual behaviour, immobilization), and/or death.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 96-h LC_{50} for *Mysidopsis bahia* exposed to glyphosate acid was 80 mg a.s./L based on nominal concentrations. The NOEC after 96 h was 32 mg a.s./L.

The validity criteria of OCSPP 850.1035 were fulfilled. The study is therefore considered valid and reliable for the regulatory risk assessment of glyphosate.

Assessment and conclusion by RMS:

This study is considered valid.

Only ten mysids were used per test concentration (instead of the recommended 20 in 2 replicates of 10, cf OCSPP 850.1035). On the other hand, 8 concentrations were tested (instead of 5 as recommended). RMS considers that the deviation still allows for reliable conclusions.

Salinity should be 20 ppt (and constant within ±2 ppt during the test). Salinity was measured in water control and 1000 (pH adjusted) mg/L test concentration, and was of 16.5 ppt. RMS considers the deviation acceptable.

The current guideline OCSPP 850.1035 recommends a pH between 7.5-8.5. The pH value at 56, 100 and 180 mg/L (pH of 6.7, 6.0 and 4.5 respectively) was below the recommended range. This deviation was negatively correlated with increasing concentrations indicating that this pH decrease was due to the test item. The mortality observed at these concentrations can be explained by the acidity of the test solution. The pH adjusted concentrations can not be considered relevant as it is not possible to have a complete dose response (only 3 concentration tested).

Given that the proposed 96 hour LC50 for Mysidopsis bahia exposed to glyphosate acid of 80 mg a.s./L and its 95% confidence interval based on nominal concentrations is between 56 and 100 mg/L, RMS considered the LC50 suitable for risk assessment.
NOEC = 32 mg a.s./L

Data point:	CA 8.2.4.2/002
Report author	[redacted]
Report year	1978
Report title	Toxicity of seven test materials to mysid shrimp *Mysidopsis bahia*
Report No	BP-78-4-032
Document No	-
Guidelines followed in study	Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OCSPP 850.1035 (2016):* *Major:* • *No analytical verification performed* • *No indication of the organisms randomisation*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed.
Acceptability/Reliability (RMS):	Not reliable

Executive Summary

The effects of seven test items, two solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45) and five liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A) on mysid shrimp, *Mysidopsis bahia*, were evaluated in a 96-hour static toxicity test. The test concentrations used for solid test items were using 3.2, 10, 32, 56, 100, 1000 mg test item/L. For the liquid test items, the concentrations used were 0.6, 1.0, 3.2, 10, 32 and 56% effluent. The test solutions were prepared using seawater. In addition, a control group was exposed to seawater without test material. There was one replicate (3.5 L glass jar) per treatment (7 jars for each solid test material and 8 jars for each liquid test material), containing each ten mysids in 3 L test solution.
Mortality was recorded in all test concentrations and the control 24, 48, 72 and 96 hours after test initiation.
For the two solid test materials (Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45) the highest mortality was 20% in the 1000 mg test item/L treatment group for both test items after 96 hours of exposure. For the liquid materials, the highest mortality was observed with Comp. #3A, BN-78-48, while the lowest mortality was obtained with Comp. #l, BN-78-46 and "Comp. #4, BN-78-49. In Comp. #3A, BN-78-48, mortality was 40% and 30% in the non-aerated and aerated test solutions of the 10%

effluent treatment group, respectively; in Comp. 5A, mortality was 0% and 10% in the non-aerated and aerated treatments of the 10% effluent treatment group, respectively. However, oxygen demand apparently contributed to the toxicity of these two samples in concentrations ≥ 32% effluent. The applicant considered the study as supportive as no analytical verification was performed and organisms randomisation was not performed or reported. RMS considers this study as not reliable (see commenting box below).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item (Description):	Glyphosate, BN-78-44 (white, crystalline solid) Glyphosate intermediate, BN-78-45 (fine, white powder) Comp. #1, BN-78-46 (clear liquid) Comp. #2, BN-78-47 (clear liquid) Comp. #3A, BN-78-48 (murky liquid) Comp. #4, BN-78-49 (clear liquid) Comp. 5A. (clear liquid)

2. Vehicle of test material and/or positive control: Dodecyl sodium sulphate (DSS)

3. Test organism:

Species:	Mysid shrimp (*Mysidopsis bahia)*
Age:	6 – 8 days old
Size:	4 – 6 mm length
Loading:	10 test individuals for 3 L test solution
Source:	In-house culture
Diet/Food:	None
Acclimation period:	48 hours prior to the test initiation
Body weight of the animals:	Not stated

4. Environmental conditions:

Temperature:	20 ± 1°C
Photoperiod:	Not stated
pH or Salinity (‰):	Glyphosate, BN-78-44, (6.4 – 8.3) Glyphosate intermediate, BN-78-45. (6.8 – 8.3) Comp. #1, BN-78-46 (8 – 20‰) Comp. #2, BN-78-47 (8 – 20‰) Comp. #3A, BN-78-48 (20 – 32‰) Comp. #4, BN-78-49 (12 – 20‰)
Dissolved oxygen:	Glyphosate, BN-78-44, (6.4 – 7.7 mg O_2/L) Glyphosate intermediate, BN-78-45. (6.4 – 7.4 mg O_2/L) Comp. #1, BN-78-46 (6.1 – 7.6 mg O_2/L) Comp. #2, BN-78-47 (6.1 – 7.4 mg O_2/L) Comp. #3A, BN-78-48 (0.4 – 7.4 mg O_2/L) Comp. #4, BN-78-49 (4.9 – 7.6 mg O_2/L) Comp. 5A. (0.3 – 7.4 mg O_2/L)
Conductivity:	Not stated

Hardness: Not stated

5. Experimental dates: Not stated

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Toxicity tests for the seven test materials were performed using 3.2, 10, 32, 56, 100, 1000 mg test item/L for the two solid test materials (Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45) and the nominal concentrations of 0.6, 1.0, 3.2, 10, 32 and 56% effluent for liquid materials (Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.). For solid test materials, appropriate amounts were added to deionised water; the pH was adjusted to 8.0, and the materials were finally diluted in seawater in the test containers to obtain appropriate concentrations. For liquid materials, the test solutions were prepared by adding appropriate volumes of test materials to seawater in the test containers: Two containers of 10% test concentration were tested for each material, one aerated and one non-aerated. In addition, a control group was exposed to seawater without test material. Salinity controls were also maintained; mysids were exposed to salinities corresponding to the lowest and highest (8 and 32 ‰) salinity occurring in any of the test concentrations.

There was one replicate (3.5 L glass jar) per treatment (7 jars for each solid test material and 8 jars for each liquid test material), containing each ten mysids in 3 L test solution.

A separate test was conducted, in which mysids were exposed to the reference toxicant dodecyl sodium sulfate under the same test conditions as for the test materials.

2. Observations: Mortality was recorded in all test concentrations and the control 24, 48, 72 and 96 hours after test initiation. Temperature was constantly maintained at 20 ± 1°C; pH-value and oxygen saturation of the test solutions were measured at test initiation and test termination.

3. Statistical calculations: The percentage of dead mysids was converted to a Probit (Finney, 1971) and the LC_{50} values were then calculated by linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} values are given below based on nominal concentrations.

Table B.9.2.4-39: Endpoints

Test materials	EC_{50} (96 h) [% effluent or mg test item/L)]
Glyphosate, BN-78-44	> 1000 mg test item/L
Glyphosate intermediate, BN-78-45	> 1000 mg test item/L
Comp. #1, BN-78-46	> 56 % effluent
Comp. #2, BN-78-47	5.6 % effluent
Comp. #3A, BN-78-48	2.8 % effluent
Comp. #4, BN-78-49	> 56 % effluent
Comp. 5A.	> 10, <32 % effluent

B. OBSERVATIONS

Clinical observations:
For the two solid test materials (Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45) the highest mortality was 20% in the 1000 mg test item/L treatment group for both test items after 96 hours

of exposure. For the liquid materials, the highest mortality was observed with Comp. #3, BN-78-48, while the lowest mortality was obtained with Comp. #l, BN-78-46 and "Comp. #4, BN-78-49.
Two of the liquid samples, Comp. #3 and Comp. 5A, had considerable oxygen demand. In test concentrations < 10% effluent, the oxygen demand did not contribute appreciably to toxicity.
In Comp. #3A, BN-78-48, mortality was 40% and 30% in the non-aerated and aerated test solutions, respectively; in Comp. 5A, mortality was. 0% and 10% in the non-aerated and aerated treatments, respectively. However, oxygen demand apparently contributed to the toxicity of these two samples in concentrations ≥ 32% effluent.

Table B.9.2.4.2-4: Lethal effects of Glyphosate, BN-78-44 and Glyphosate intermediate, BN-78-45 on *Mysidopsis bahia*

Test items [mg/L] →	**Control**	**3.2**	**10**	**32**	**56**	**100**	**1000**
Glyphosate, BN-78-44							
Mortality (24 h) [%]	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	10	0
Mortality (96 h) [%]	0	0	10	0	0	10	20
Glyphosate intermediate, BN-78-45							
Mortality (24 h) [%]	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	0	10	10
Mortality (96 h) [%]	0	0	10	0	0	10	20

Table B.9.2.4.2-5: Lethal effects of Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A. on *Mysidopsis bahia*

Test items [% effluent] →	Control	0.6	1.0	3.2	10	10 AE	32	56
Comp. #1, BN-78-46								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	10	0	0	0	0	0
Mortality (96 h) [%]	0	0	10	0	10	0	0	10
Comp. #2, BN-78-47								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	20	10	30	100
Mortality (96 h) [%]	0	0	10	10	70	70	90	100
Comp. #3A, BN-78-48								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	30	20	0	40	20	100	100
Mortality (96 h) [%]	0	30	20	20	40	30	100	100
Comp. #4, BN-78-49								
Mortality (24 h) [%]	0	0	0	0	0	0	0	0
Mortality (48 h) [%]	0	0	0	0	20	10	20	0
Mortality (96 h) [%]	0	0	0	0	20	20	20	30
Comp. 5A								
Mortality (24 h) [%]	0	0	0	10	0	0	0	0
Mortality (48 h) [%]	0	0	0	10	0	0	100	100
Mortality (96 h) [%]	10	20	20	10	0	10	100	100

AE = aerated

Table B.9.2.4.2-6: Lethal effects of the toxic reference dodecyl sodium sulfate on *Mysidopsis bahia*

Test items [mg/L] →	Control	6	8	10
Mortality (24 h) [%]	0	0	20	20
Mortality (48 h) [%]	0	0	20	20
Mortality (96 h) [%]	0	30	60	70

The following points deviated from OCSPP 850.1035 Mysid Acute Toxicity Test (October 2016):

- Analytical confirmation of dissolved test concentrations were not performed.

The validity criteria of OCSPP 850.1035 guideline (2016) are the following:

- All test vessels were identical - achieved
- Individual test organisms were randomly assigned to test vessels - no information in the report.
- A dilution water control was included in the test - achieved
- Not more than 10% of the organisms in the dilution water control showed signs of disease, stress (*e.g.*, discoloration, unusual behaviour, immobilization), and/or death - achieved

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of seven glyphosate-related test items on *Mysidopsis bahia* were studied in a static acute toxicity test. The EC_{50} (96 h) for *Mysidopsis bahia* exposed to Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and were > 56, 5.6, 2.8 and > 56% effluent respectively. The EC_{50} (96 h) for Comp. 5A was found to be between 10 and 32% effluent. For the test items Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45, no EC_{50} were calculated since the effects on mysid shrimps were low at the highest test concentration.

No analytical verification was performed and organism randomisation was not performed or reported. The study is therefore considered to be supportive for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

This study was not submitted/assessed in RAR 2015.
This study was not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed).
Only one replicate per treatment was used (where minimum of 2 is required).
The shrimps were 6-8 days old (instead of < 24h).
Temperature was of 20°C (instead of 25°C).
Salinity was heterogenous among test concentrations (8-32 ppt, instead of 20 as recommended).
Dissolved oxygen decreased to very low levels for some of the test items (at highest concentrations). However this was not observed for all the test items at equivalent concentrations. So this decrease in oxygen levels is not treatment related.
No analytic verification was performed and therefore dose verification is impossible.
The pH values were available. A pH value of 6.4 minimum was measured. On the basis of the current knowledge, it is obvious that such concentrations (of technical glyphosate) would have decreased the pH in the test media to a far lower value. The dosing is doubtful and the effect observed in this study are likely to be underestimated.
Besides endpoints expressed as %effluent cannot be used in the risk assessment.

RMS considers this study as not reliable.

Data point:	CA 8.2.4.2/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Acute toxicity to larvae of the Pacific oyster (*Crassostrea gigas*)
Report No	AB0503/G
Document No	-
Guidelines followed in study	EPA FIFRA, Subdivision E, Guideline 72-3 ASTM (1989) E724/9-85-012 (OPPTS 850.1055)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from OPPTS 850.1055 (1996): none.*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary

The effects of glyphosate acid to pacific oyster (*Crassostrea gigas*) was evaluated in a 48-hour static toxicity test conducted with nominal test concentrations of 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg Glyphosate acid/L. Furthermore, a dilution water control was tested. To each test vessel 0.535 mL inoculum containing 22 embryos/ mL was added. For each test item concentration 2 replicates and for the control 4 replicates were tested. The number of normal and abnormal larvae was counted after 48 h. Dissolved oxygen and pH were measured at test start and test end, while the temperature was measured daily. The salinity was measured in the dilution water control and in the 180 mg/L test solution and the density of the embryo solution was determined by electronic particle counting before test start. Test item concentrations were verified by HPLC at 0 and 48 hours. Mean measured concentrations ranged from 91 to 100% of nominal concentrations.

The reduction of oyster development was assessed with a parametric and a non-parametric test which both indicated no significant reduction of development up to nominal concentrations of 32 mg test item/L. The LC_{50} (48 h) for *Crassostrea gigas* was 40 mg a.s./L (nominal). The NOEC after 48 h was 32 mg a.s./L. All validity criteria according to OPPTS 850.1055 were fulfilled. The study is therefore considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%

Density:	Not stated
2. Vehicle of test material/media:	Vehicle: Dilution water
3. Test organism:	
Species:	Pacific oyster (*Crassostrea gigas*), Brood stock batch OY17
Age:	Embryos, approx. 15 minutes after fertilisation
Source:	In-house culture originally obtained from Guernsey Sea Farms, Parc Lane, Vale, Guernsey, Channel Islands, UK
Density of embryo solution at test start:	22 embryos/mL
4. Environmental conditions:	
Temperature:	19.4 – 20.5°C
pH:	5.6 – 8.1
Dissolved oxygen:	7.0 – 7.8 mg O_2/L
Salinity:	31.0 – 31.5‰
5. Experimental dates:	April 23, 1996 to April 25, 1996

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test was performed using nominal concentrations of 3.2, 5.6, 10, 18, 32, 56, 100 and 180 mg glyphosate acid/L prepared using natural sea water, filtered through 0.2 µm with adjusted salinity (32 ±2‰). In addition, a control was exposed to the test medium without test substance or other additives.

The test was conducted 48 h in a static test setup in 250 mL glass beakers with loose fitting lids. There were two vessels per test concentration and four for the control group, each containing 0.535 mL embryo solution with an embryo density of 22 embryos/mL (determined in three additional inoculated vessels). At test end, the test media were mixed, and 20 mL removed and fixed with 1 mL buffered formalin. The number of normal and abnormal larvae was counted. Larvae were defined as normal, if the bivalve shell was fully formed.

2. Observations: The number of normal and abnormal larvae was counted at test end in triplicate in 1 mL subsamples using an inverted microscope. The pH-value and the oxygen saturation were measured at test start and test end. The temperature was measured daily in one replicate of each test solution. The salinity was measured in the dilution water control and in the 180 mg/L test solution. The density of the embryo solution was determined by electronic particle counting before test start. Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis at test start and test end.

3. Statistical calculations: The EC_{50} value was calculated using Stephan's method. The significance of reduction in normal development was assessed using the Students t-test with Bonferroni adjustment (parametric) and Wilcoxon rank sum test (non-parametric).

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value and the NOEC are given below based on nominal concentrations.

Table B.9.2.4.2-7: Endpoints

Endpoints	Glyphosate acid [mg/L]
EC_{50} (48 h) (95% CL)	40 (36 – 45)
NOEC (48 h)	32

Analytical data: The mean measured concentrations of glyphosate acid ranged from 91 to 100% of nominal values. As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.4.2-8: Analytical results

Nominal concentration of glyphosate acid [mg/L]	Measured concentration of glyphosate acid [mg/L]		Mean measured concentration of glyphosate acid [mg/L]	% of nominal
	0 h	48 h		
Dilution water control	< 0.01	<0.01	<0.01	-
3.2	3.0	2.7	2.9	91
5.6	5.7	5.1	5.4	96
10	10	8.8	9.4	94
18	18	16	17	94
32	32	30	31	97
56	56	52	54	96
100	100	94	97	97
180	180	170	180	100

B. OBSERVATIONS

The reduction of oyster development was assessed with two statistical methods. The parametric test (Students t-test with Bonferroni adjustment) calculated a non significant reduction at nominal concentrations up to 32 mg/L. As these data were non-parametric, the Wilcoxon rank sum test for non-parametric data was conducted, which also indicated no significant development reduction up to nominal concentrations of 32 mg test item/L.

The results of the test are depicted in the following tables.

Table B.9.2.4.2-9: Effects of glyphosate acid to *Crassostrea gigas*

Nominal concentration of glyphosate acid [mg/L]	Number of normal / abnormal oysters after 48 h				Mean normal oysters [%]	Reduction [%]
	A	B	C	D		
Control	43/0	36/4	46/1	45/2	103	-
3.2	42/1		37/2		95	8
5.6	43/0		41/2		100	3
10	45/1		42/1		105	0
18	38/1		38/3		90	13
32	41/4		37/4		91	12
56	12/21		9/25		27	74*
100	0/26		0/29		0	100*
180	0/11		0/12		0	100*

*significant reduction

All validity criteria according to OPPTS 850.1055 were fulfilled, as mortality/ aberrant development in control group did not exceed 30%, dissolved oxygen concentration was ≥ 60% of air saturation and embryos were ≤ 4 h old at test start.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In conclusion, the LC_{50} (48 h) for *Crassostrea gigas* exposed to glyphosate acid was 40 mg a.s./L (nominal). The NOEC after 48 h was 32 mg a.s./L, based on nominal test concentrations.

The study is considered to be valid and reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:

This study is considered valid.

No particular recommendation concerning the appropriate pH is given in the guideline OPPTS 850.1055 (Bivalve Acute Toxicity Test (Embryo-Larval)). Some recommendations are however available in the existing guideline for the older lifestage of oyster OPPTS 850.1025 (i.e. pH between 7.5 and 8.5). The pH values in this study were between 5.6 and 8.1. The pH at the highest concentration (pH 5.6 at 180 mg/L) was below this range. Nevertheless the pH seems appropriate for this species in all other tested concentrations. Therefore, the effects observed in this study does not seem to be "pH dependant".

EC50 (48 h) for *Crassostrea gigas* exposed to glyphosate acid = 40 mg a.s./L (nominal).
NOEC = 32 mg a.s./L

Data point:	CA 8.2.4.2/004
Report author	[redacted]
Report year	1985
Report title	Acute Toxicity of Roundup (Technical) to Atlantic Oyster (*Crassostrea virginica)*
Report No	BN-73-79
Document No	-
Guidelines followed in study	Woelke, C. E. - "Measurement of Water Quality with the Pacific Oyster Bioassay." Water Quality Criteria, ASTM Spec. Tech. Publ. 416, Am. Soc. Testing Mats, 1967, p. 112-120.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from OPPTS 850.1055 (1996):* • *No information about the dissolved oxygen concentration.* • *No analytical verification.*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Not reliable

Executive Summary

The effects of glyphosate technical on the normal embryonic development of the Atlantic oyster (*Crassostrea virginica*) were evaluated in a 48-hour static toxicity test. The test was performed using nominal concentrations of 0.75, 1.0, 2.4, 4.9, 7.5 and 10 mg glyphosate/L in triplicates. In addition, a control with test medium without test substance was tested.

The test was performed in 500 mL volumetric flasks, containing each 300 mL test solution, in which 15000 newly fertilised oyster eggs (at two-cell stage) were introduced for each test concentration and control. The test flasks were incubated for 48 hours at 25°C. The salinity of the test solutions was measured at test initiation to range between 26 – 28‰ at test initiation.

At the end of this period cultures were sieved and larvae were preserved in 5% formalin for microscopic examination to determine the percentage of fertilized eggs that had developed to a normal morphological stage.

Compared to the untreated control, no adverse effects of glyphosate on the normal embryonic development of oysters were observed up to the highest concentration tested (10 mg glyphosate/L). The EC_{50} and the NOEC were therefore determined to be > 10 mg/L and ≥ 10 mg/L, respectively. The applicant considered the study as supportive (no analytical verification).

RMS considered this study as not reliable (see commenting box).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	CP-67573
Purity:	96.7%

2. Test organism:

Species:	Atlantic oyster (*Crassostrea virginica*)
Age:	Fertilised eggs
Size:	Not stated
Loading:	50.000 fertilized eggs/L
Source:	U. S. Bureau of Commercial Fisheries Shellfish Research Laboratory in Milford
Diet/Food:	None
Acclimation period:	Sexually mature Atlantic oysters were collected from Milford harbour and held at the BCF' Shellfish Laboratory in filtered sea water for 7 days at a temperature of 22C.

3. Environmental conditions:

Temperature:	25 °C
Photoperiod:	Not stated
Salinity	26 – 28‰ (at test start)
Dissolved oxygen:	Not stated
Conductivity:	Not stated

4. Experimental dates: Not mentioned

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The static acute toxicity test was performed using nominal concentrations of 0.75, 1.0, 2.4, 4.9, 7.5 and 10 mg glyphosate/L in triplicates. In addition, a control with the test medium (without test substance) was tested under the same conditions as in the test groups. Ten hours prior to the test initiation, mature oysters were placed in a Pyrex tray filled with ultraviolet-light-treated water for eggs laying. About 30 minutes before spawning was desired, the water temperature was raised to 30°C and a sperm suspension from a sexually mature, sacrificed male oyster was added to the water. The combination of increased temperature and sperm induced one or more of the female oysters to spawn. Eggs from a single female were selected for use in the bioassay and the number of eggs/unit volume was determined by sampling the sperm-egg suspension. The test was performed in 500 mL volumetric flasks, containing each 300 mL test solution with a salinity of 26 – 28‰, in which 15000 newly fertilised oyster eggs (at two-cell stage) were introduced for each test concentration and control. The test flasks were incubated for 48 hours at 25°C. At the end of' this period cultures were poured through a 37 µm sieve to obtain samples containing about 200 larvae and samples were preserved in 5% formalin for microscopic examination.

2. Observations: Quantitative samples were taken 48 hours after test initiation to determine the percentage of the fertilized eggs that had developed to a normal morphological stage (straight-hinged veliger larvae).

3. Statistical calculations: The concentrations tested and the corresponding observed percent normal development were transformed to log and Probit, respectively. The EC_{50} values were predicted using a linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} and NOEC values given below are based on nominal concentrations.

Table B.9.2.4.2-10: Endpoints

Endpoints	Test item [mg/L]
EC_{50} (48 h)	> 10
NOEC (48 h)	≥ 10

B. OBSERVATIONS

Compared to untreated control, no adverse effects of glyphosate on the normal embryonic development of oysters were observed up to the highest concentration tested (10 mg test item/L).

Table B.9.2.4.2-11: Percentage normal development of Atlantic oyster larva exposed to glyphosate for 48 hours

Glyphosate [mg/L]	Control	0.75	1.0	2.4	4.9	7.5	10.0
Normal embryonic development [%]	> 90	> 90	> 90	> 90	> 90	> 90	> 90

Results showed that glyphosate did not adversely affect the normal development of Atlantic Oyster larvae.

The validity criteria according to OPPTS 850.1055 are the following:

- The mortality/aberrant development in control group should not exceed 30% - achieved
- The dissolved oxygen concentration should be ≥ 60% of air saturation – no information in the report
- The embryos should be ≤ 4 h old at test start – two cell stage embryos were used.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In an acute toxicity test, Atlantic Oysters (*Crassostrea virginica*) were exposed to glyphosate technical for 48 hours. The EC_{50} and the NOEC were therefore determined to be > 10 mg a.e./L and ≥ 10 mg a.e./L, respectively.

Since no analytical verification was performed, the study is considered to be supportive and not considered reliable for the regulatory risk assessment for glyphosate.

Assessment and conclusion by RMS:
This study was not submitted/assessed in RAR 2015.

In an acute toxicity test, Atlantic Oysters (*Crassostrea virginica*) were exposed to glyphosate technical for 48 hours. The EC50 and the NOEC were reported to be > 10 mg/L and = 10 mg/L, respectively.

This study was not conducted under GLP/Officially recognised testing facilities (GLP was not compulsory at the time the study was performed).
No information about the dissolved oxygen concentration is available (validity criteria).
No analytic verification was performed and therefore dose verification is impossible.
The pH values are not available. Other water parameters are also lacking.

No robust endpoint can be derived from this study.

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

B.9.2.5.1. Reproductive and development toxicity to Daphnia magna

Data point:	CA 8.2.5.1/001
Report author	[redacted]
Report year	1999
Report title	Glyphosate acid: Chronic toxicity to *Daphnia magna*
Report No	AF0497/B
Document No	-
Guidelines followed in study	OECD 202, Part II, Reproduction Test (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one *Daphnia* per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 *Daphnia* were exposed to test medium without test substance (blank control). The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 21 days. The test *Daphnia* were fed daily with cultured algae (*Chlorella vulgaris*).
Mortality of P_0 generation of *Daphnia* and observation for the presence of alive and dead offspring (termed F_1 generation) were recorded daily in each test vessel. At the end of the test, the length of each surviving P_0 *Daphnia* was measured.
The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer of the P_0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21.

The mean measured concentrations of glyphosate acid in the new test solutions ranged from 100 to 104% of the nominal values. The mean measured concentrations in the old test solutions ranged from 96 to 104% of the nominal values. Therefore, the results are based on nominal glyphosate acid concentrations. The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid was 50 mg/L based on nominal concentration. All validity criteria according to the pertinent OECD 211 guideline were fulfilled.
The authors concluded that the overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid was 50 mg/L based on nominal concentration.
The applicant concluded that the NOEC of 50 mg a.s./L for immobility and the NOEC of 12.5 mg a.s./L for reproduction, are the most reliable and relevant endpoints to be considered in the regulatory risk assessment.
RMS concluded that 21-day NOEC for the reproduction of Daphnia magna exposed to glyphosate acid is 12.5 mg a.s./L (nominal).
The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Lot/Batch #:	P30
Purity:	97.6%

2. Vehicle of test material/media: Dilution water: Dilution water

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	1 organism per vessel (glass beakers containing 80 mL test solution)
Source:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	19.4 to 20.2°C
pH:	3.67-8.02 (new solutions) 3.46-8.00 (old solutions)
Dissolved oxygen:	9.2-9.2 mg O_2/L (dilution water, new) 8.8-9.2 mg O_2/L (test solutions, old)
Conductivity:	572-617 mg/L µS/cm (test solutions)
Hardness:	202.7-218.3 mg $CaCO_3$
Photoperiod:	16 hours light /8 hours dark, 20 minute dawn and dusk transition period; 480 lux

5. Experimental dates: November 16, 1998 to December 07, 1998

B. STUDY DESIGN AND METHODS

Experimental treatments: The lethal and sub lethal effects of glyphosate acid on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. Ten replicates of one *Daphnia* per concentration were exposed to 12.5, 25, 50, 100, and 200 mg a.s./L nominal concentrations. In addition, 10 x 1 *Daphnia* were exposed to test medium without test substance (blank control). The

Daphnia were randomly placed into the test beaker and exposed to the test item for 21 days. The test *Daphnia* were fed daily with cultured algae (*Chlorella vulgaris*).

A primary stock solution of 200 mg a.s./L was prepared on day 0 by dissolving 400 mg test item in 2000 mL of dilution water. On days 2, 4, 7, 9, 11, 14, 16, and 18 a primary stock solution of 100 mg a.s./L was prepared by dissolving 200 mg test item in 2000 mL dilution water. The test solutions were prepared by the addition of appropriate aliquots of the stock solutions to dilution water. At each renewal of the test solutions, the surviving P_0 generation of *Daphnia* were transferred to the new solutions. The F_1 generation of *Daphnia* were removed from each vessel and counted. The numbers of alive and dead F_1 *Daphnia* were recorded.

2. Observations: Mortality of P_0 generation of *Daphnia* and observation for the presence of alive and dead offspring (termed F_1 generation) were recorded daily in each test vessel. At the end of the test, the length of each surviving P_0 *Daphnia* was measured.

The pH was measured in each newly prepared test solution. The pH and dissolved oxygen concentration of two of the replicates of the old test solutions were measured after transfer of the P_0 generation of daphnids. Temperature measurements were recorded daily by means of a thermometer and hourly automatically. The concentration of glyphosate acid in the test solutions was determined on days 0, 2, 7, 9, 14, and 16. Old solutions were analysed on days 2, 7, 9, 14, and 21.

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: The reproduction and length data for each individual P_0 generation daphnid were entered into electronic data files and analysed using statistical procedures contained in the Brixham Environmental Laboratory computer programs 'STATS' (version 4.10) and 'EPA' (version1.04).

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate acid in the new test solutions ranged from 100 to 104% of the nominal values. The mean measured concentrations in the old test solutions ranged from 96 to 104% of the nominal values. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results.

Table B.9.2.5.1-1: Analytical measurements

Nominal concentration (mg glyphosate acid/L)	Mean measured (new solutions) mg/L	Mean measured (old solutions) mg/L	% of nominal of overall mean measured concentrations
Control	-	-	-
12.5	13 (104%)	12 (96%)	100
25	25 (100%)	25 (100%)	100
50	50 (100%)	52 (104%)	102
100	100 (100%)	102 (102%)	101
200	200 (100%)	200 (100%)	100

The 21-day EC_{50} and NOEC values (based on nominal concentrations) are given below:

Table B.9.2.5.1-2: Toxicity values for *Daphnia magna*

Mortality	
21-day EC_{50}	100 (95 % confidence interval 77-142)
21-day NOEC	50
21-day LOEC	100
Maximum allowable toxicant concentration (MATC)	71
Reproduction	
21-day NOEC	100 (considered 12.5 by RMS)
21-day LOEC	200 (considered 25 by RMS)
Maximum allowable toxicant concentration (MATC)	141 (MATC not considered by RMS)
Length	
21-day NOEC	100
21-day LOEC	200
Maximum allowable toxicant concentration (MATC)	141
Overall result	
21-day NOEC	50 (considered 12.5 by RMS)
21-day LOEC	100(considered 25 by RMS)
Maximum allowable toxicant concentration (MATC)	71 (MATC not considered by RMS)

B. OBSERVATIONS

In the dilution water control and test concentrations up to and including 100 mg a.s/L all surviving P_0 *Daphnia* generation had released their first offspring by day 10. There was no reproduction at the concentration of 200 mg a.s./L due to mortality of the P_0 *Daphnia*.

The effects of glyphosate acid on *Daphnia magna* mortality and reproduction are shown below.

Table B.9.2.5.1-3: Effects of glyphosate acid on *Daphnia magna* mortality and reproduction after 21 days of exposure

Nominal concentration (mg a.s./L)	Mean adult mortality [%]	Total number of off-spring per parent	Total offspring	Mean adult length [mm]
Control	10	108± 20	1028	4.28
12.5	0	100±21	1003	4.40
25	**0**	**84±12***	**840**	**4.31**
50	0	91±18	912	4.31
100	50	109±23	763	3.81
200	100	-	-	[A]

[A] mortality before day 21
* Statistically significant difference

All validity criteria according to OECD 211 were fulfilled, as immobility of adult daphnids was ≤20% in control groups and number of off-spring was >60 for the duration of the exposure.

The results of the statistical analysis for ECx calculations are reported in the position paper summarised below. Applicant conclusion followed by assessment of RMS are presented after this position paper.

Data point	CA 8.2.5.1/009
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL6535/B on the chronic toxicity of Glyphosate acid technical to *Daphnia magna* under static-renewal conditions
Report No	110054-012
Document No	-
Guidelines followed in study	OECD Guideline No. 202 (1984)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: BL6535/B
Author: [redacted]
Substance: Glyphosate acid technical
Title: Glyphosate acid: Chronic toxicity to *Daphnia magna*
Completion date: 07-12-1998
Test guideline(s): OECD 202, Part II Reproduction Test (1984)
GLP: Yes
Testing facility: Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon, UK
Sponsor: ZENECA Agrochemicals, Fernhurst Haslemere, Surrey, UK

B. STUDY DESIGN

Dates of work: August 2020

The study BL6535/B ([redacted] 1999) was statistically evaluated for the effects of glyphosate acid on the organism *Daphnia magna*. The organisms were exposed for 21 days to the following concentrations of glyphosate acid 12.5, 25, 50, 100, and 200 mg test item/L (nominal). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:

In order to derive Effect Concentrations that have 10 and 20% effects for length of the test subjects (EC_{10} and EC_{20}), a 3-parametric normal CDF analysis with Levenberg-Marquardt optimization using non-linear regression was performed. For immobility, a probit analysis with linear maximum likelihood regression was used.
Due to a lack of dose-response, no model could be fitted for reproductive data (cumulative offspring per survived parent).

All statistical evaluations were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

For reproduction, expressed as cumulative offspring per survived parent after 21 days, no clear dose-response relationship could be observed. The second highest tested concentration 100 mg/L resulted in the lowest decrease in reproduction of 1.4%. Due to 100% immobility at the highest test concentration (200 mg/L), there was no reproduction at this level. The test concentrations 50 mg/L and 25 mg/L resulted in a decrease in reproduction of 17% and 22%, respectively. Therefore, no statistical model could be fitted to the data with reliable EC_{10} and EC_{20} calculations.

For immobility, the model did not show a good fit due to an insufficient dose-response relationship, in addition to 10% mortality in the control. The second lowest test concentration 25 mg/L showed a 10% decrease in immobility, while the next highest test concentration 50 mg/L showed no decrease in immobility.

The parameters for the probit model are estimated as slope b: 3.86131; Intercept a: -7.66585. The parameters for goodness of fit are: $Chi^2(3) = 10.18$; $p(Chi^2 = 0.017$; $F(1,3)=4.361$, $p(F) = 0.128$. as $p(F) > 0.05$, no statistically significant concentration-response can be determined by the model.

Nevertheless, it can be estimated that the EC_{20} is in the range of 50 – 100 mg/L for immobility.

For length the second highest test concentration 100 mg/L resulted in a 11% decrease compared to the control, while no decrease was observed in the lower test concentrations. Since no surviving daphnia were observed in the highest test concentration 200 mg/L, no decrease in length was calculated.

The parameters for the 3-parametric normal CDF model are estimated as b0: 53.004, b1: 1.975, and b2: 0.223.
According to the statistical parameters; $F(2, 3) = 20.802$; $p(F) = <0.001$; $R^2 = 0.479$ the EC_{10} for immobility calculations should be considered valid. After non-linear regression no lack of fit was detected for the function ($p(F|Lack of Fit) = 0.316$ for length.

The obtained EC_{10} and EC_{20} values for the effect of Glyphosate acid on reproduction, immobility, and length of *Daphnia magna* are presented in the table below.

Table B.9.2.5.1-4: Re-calculated EC_{10} and EC_{20} values based on nominal concentrations

Endpoint (21 days)	Glyphosate acid technical [mg a.s./L]		
	Cumulative offspring per survived parent	Immobility	Length
EC_{10} (95% CI)	12.5 – 100	n.d.	94.47 (83.22 – > 100)
EC_{20} (95% CI)		50 – 100	> 100

* = due to insufficient dose-response relationship true values could be deviating from calculated values
CI = confidence interval
n.d. = not detected

III. CONCLUSION

Assessment and conclusion by applicant:

The overall 21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid was 50 mg a.s./L based on nominal concentration. The EC50 was determined to be 100 mg a.s./L.

In the RAR 2015, the RMS considered the nominal NOEC to be 12.5 mg a.s./L based on statistical difference at the next higher test concentration.

The study is considered to be valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10} and EC_{20}, was performed (Position Paper No. CA 8.2.5.1/009) to fulfil the data requirements according to regulation EU 283/2013.

Re-calculated EC_{10} and EC_{20} values based on nominal test concentrations:

Endpoint (21 days)	Glyphosate acid technical [mg a.s./L]		
	Cumulative offspring per survived parent*	Immobility	Length
EC_{10} (95% CI)	12.5 – 100	n.d.	94.47 (83.22 – 107.26)
EC_{20} (95% CI)		50 - 100	> 100

* = due to insufficient dose-response relationship true values could be deviating from calculated values
CI = confidence interval
n.d. = not detected

Thus, the NOEC of 50 mg a.s./L for immobility and the NOEC of 12.5 mg a.s./L for reproduction, are the most reliable and relevant endpoints to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

This study is considered valid.

The study was performed according to OECD 202, Part II. The relevance of this study is reassessed using the criteria of the current guideline OECD 211 (reassessment already made in RAR 2015).

According to the current guideline OECD 211, the pH should be within the range 6 – 9. The pH at the highest concentration (pH 3.46 at 200 mg/L) was below this range. This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of glyphosate acid). The high mortality observed at this concentration can be (at least partly) explained by the acidity of the test solution.
Nevertheless the pH seems appropriate in all other tested concentrations (within the recommended range at 0, 12.5, 25, 50 mg/L and only slightly below the recommended range, pH of 5.34 at 100 mg/L). Therefore, the effects observed at intermediate concentrations do not seem to be "pH dependant".

As already highlighted in RAR 2015, since daphnids were held individually in the test vessel, it is possible to determine the exact number of offspring per parent and therefore a statistical evaluation according to the criteria of OECD 211 is possible. It was proposed to consider the significant effects at 25 mg/L and to recommend a NOEC for reproduction at 12.5 mg a.s./L based on nominal concentration. RMS agrees with this reassessment. Indeed, even if the dose response is not perfect, RMS considers that effects at 25 can not be ignored considering also that 16% reduction of total number of offspring per parent or 11.3% reduction in total offspring is observed at 50 mg/L.

21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid = 12.5 mg a.s./L (nominal).

No suitable EC10 could be set for immobility. EC10 for length was set to 94.47 mg/L.
No ECx values for reproductive data (cumulative offspring per survived parent) could be set due to the lack of dose-response.

The NOEC was retained as the suitable endpoint to consider in risk assessment.

Data point:	CA 8.2.5.1/002
Report author	[redacted]
Report year	1995
Report title	*Daphnia magna*, Reproduction Test with Glyfosaat
Report No	141874
Document No	-
Guidelines followed in study	OECD Guideline 202 ECC Draft Guideline XI/681/86 "Prolonged Toxicity Study with *Daphnia magna*: Effects on Reproduction"
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid with restriction (pH issue)

Executive Summary

The effects of glyphosate (glyfosaat) on *Daphnia magna* were evaluated in a 21-day reproduction test under semi-static conditions. The reproduction test was performed using six nominal test concentrations (5, 10, 18, 32, 56 and 100 mg test item/L) and a control. 10 replicates with one daphnid each were prepared per test concentration and 20 replicates with one daphnid each for the control.

The number of living, immobilised and dead parental *Daphnia magna* was observed on a daily basis. In addition, the presence of eggs in the brood pouch was observed on every workday. For the F1 generation, the appearance of the first brood was recorded. Every workday, the number of newborn daphnids were counted and the condition of the young recorded. The presence of eggs, which did not hatch was recorded, when observed. Incidental mortality was equally recorded, when occurred.

There was no test substance related mortality of parental daphnids at any test concentrations. The average numbers of offspring per parent at concentrations up to and including 56 mg/L were > 90% when compared to the control group. The average number of offspring at 100 mg/L ranged from 54 to 74 % when compared to the controls. Statistical analysis demonstrated significant reduction of reproductive capacity of *Daphnia magna* at 100 mg/L. The authors concluded that the EC_{50} for parental immobility and reproduction were both calculated to be > 100 mg a.e./L (nominal). The overall no observed effect concentration (NOEC) was 56 mg a.e./L based on nominal concentrations. All validity criteria according to the pertinent OECD 211 guideline were fulfilled. The study is considered to be

valid. RMS considered that although there might be an effect of pH at the highest concentration, the observed difference is substantial and should have no major impact on the results.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96%

2. Vehicle of test material/media: Dilution water (M4 medium)

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	1 daphnid per 50 mL test medium
Source:	In-house culture
Diet/Food:	*Chlorella pyrenoidosa* at each solution renewal

4. Environmental conditions:

Temperature:	19.5- 21.0°C
Photoperiod:	16 hours light / 8 hours dark, 600 lux
pH:	7.7 – 8.8 (control), 5.2 – 5.7 (100mg test item/L)
Dissolved oxygen:	> 8.9 mg O_2/L, (5.9 – 7.6 mg O_2/L on day 21 only)
Conductivity:	Not stated
Hardness:	250 mg $CaCO_3$/L

5. Experimental dates: May 5, 1995 to May 29, 1995

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A 21-day reproductive toxicity test was conducted under semi-static conditions (renewal of test medium three times a week). *Daphnia magna* was exposed to nominal concentrations of 5, 10, 18, 32, 56 and 100 mg test item/L in ISO-medium (M4). In addition, a control group was exposed to test medium without test substance. Ten glass vessels (80 mL vessels containing 50 mL test medium each) were used per treatment group for the test item and 20 vessels for the control group. One daphnid was exposed per replicate (vessel).

2. Observations: The number of living, immobilised and dead parental *Daphnia magna* was observed on a daily basis. In addition, the presence of eggs in the brood pouch was observed on every workday.

For the F1 generation, the appearance of the first brood was recorded. Every workday the number of young newborn daphnids was counted and the condition of the young recorded. The presence of unhatched eggs was recorded, when observed. Incidental mortality was equally recorded, when occurred.

The pH-values and the oxygen saturation were measured at test initiation and just before the renewal of the test media in all treatments. The temperature was controlled at each renewal in one of the control vessels and on a daily basis in the climate room.

Analytical control measurements were performed by mean of HPLC analysis using samples taken from all test concentrations on day 0 for the freshly prepared solutions. For the aged test media, samples were taken from 3 representative test concentrations.

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Data were statistically tested using a mean comparison test (Williams' t-Test; $\alpha = 0.05$). EC_{50} (immobilisation) and the EC_{50} (reproduction) were estimated.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed on samples of representative test concentrations. Recoveries ranged from 104% to 118% relative to nominal concentrations for test concentrations > 10 mg/L. Therefore, endpoints are based on nominal concentrations. At 5 and 10 mg/L recovery of glyphosate was significantly higher than nominal (> 120%). The actual concentrations did not decrease significantly during the periods between renewal (48 or 72 hours).

Table B.9.2.5.1-5: Analytical results

	mg glyphosate/L						
Nominal concentration	**Control**	**5**	**10**	**18**	**32**	**56**	**100**
Day 0	-	8.47	12.0	21.3	32.2	58.7	100
Day 3 (old)	-		14.3		35.7		110
Day 7 (fresh)	-		19.1		36.3		112
Day 14 (fresh)	-		16.7		36.7		111
Day 21 (old)	-				34.1	58.7	106
Mean measured over 21 d study		8.4	15.5	21.3	35.2	58.7	108.2
% of nominal	-	169	155	118	110	104	108

The 21-day EC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.5.1-6: Endpoints

Endpoints	Glyphosate [mg/L] Nominal concentrations	Glyphosate [mg/L] Mean measured concentrations
EC_{50} (21 days) for parental immobility	> 100	> 108
EC_{50} (21 days) for reproduction	> 100	> 108
Overall LOEC	> 100	> 108
Overall NOEC	56	59

B. OBSERVATIONS

There was no test substance related mortality of parental daphnids at any test item concentration. The average numbers of offspring per parent at concentrations up to and including 56 mg/L were > 90 % when compared to the control. The average number at 100 mg/L ranged from 54 to 74% when compared to the control. Statistical analysis shows significant reduction of reproductive capacity of *Daphnia magna* at 100 mg/L.

Table B.9.2.5.1-7: Chronic toxicity of glyphosate to *Daphnia magna*

	Control	Glyphosate [mg/L]					
		5	**10**	**18**	**32**	**56**	**100**
Immobilisation of adults after 21 d [%]	5	20	0	0	0	10	20
Cumulative mean number of living young at day 21	133	145	147	151	158	160	91.7*
mean living young compared to controls [%]	-	109	111	114	119	120	69

* Statistically significantly lower than control

All validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live offspring produced per parent animal surviving at the end of test was ≥60.

The results of the statistical analysis for ECx calculations are reported in the position paper summarised below. Applicant conclusion followed by assessment of RMS are presented after this position paper.

Data point	CA 8.2.5.1/010
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 141874 on the chronic toxicity of Glyphosate to *Daphnia magna* under static-renewal conditions
Report No	110054-013
Document No	-
Guidelines followed in study	OECD Guideline No 202 (1984), EEC Draft guideline XI/681/86, Version 4 (1986)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number:	141874
Author:	[redacted]
Substance:	Glyphosate
Title:	*Daphnia magna*, Reproduction test with Glyfosaat
Completion date:	11-07-1995
Test guideline(s):	OECD 202, Part II Reproduction Test (1984), EEC Draft guideline XI/681/86, Version 4 (1986)
GLP:	Yes
Testing facility:	NOTOX B.V., 's-Hertogenbosch, The Netherlands
Sponsor:	Agrichem B.V., AG Oosterhout, The Netherlands

B. STUDY DESIGN

Dates of work: August 2020

The study 141874 [redacted] 1995) was statistically evaluated for the effects of glyphosate on survival and reproductive performance in the organism *Daphnia magna.* The organisms were exposed for 21 days to the following concentrations of glyphosate 5, 10, 18, 32, 56, and 100 mg test item/L (nominal). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive Effect Concentrations that have 10 and 20% effects on reproduction (cumulative offspring per survived parent) of the test subjects (EC_{10} and EC_{20}), a 3-parametric normal CDF analysis with Levenberg-Marquardt optimization using non-linear regression was performed. For immobility, a probit analysis with linear maximum likelihood regression was used.

All statistical evaluations were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

For reproduction, expressed as cumulative offspring per survived parent at 21 days, no clear dose-response relationship was observed for the test item. While only the highest test concentration of 100 mg/L resulted in a decrease of 31% in the reproduction, the test concentrations of 5 to 56 mg/L resulted in an increase of reproduction compared to the control. The second highest test concentration 56 mg/L gave an increase in reproduction of 21%. Therefore, no suitable model could be fitted to the data and no reliable EC_{10} and EC_{20} values could be calculated. The EC_{10} and EC_{20} are estimated to be in the range of 56 to 100 mg a.e./L for reproduction.

For immobility, no effect was observed after a 21-day exposure to glyphosate. The parameters for the probit model are estimated as slope b: - 0.72325; Intercept a: - 0.65709.

Statistical parameters for goodness fit are: $Chi^2(4) = 1.18298$; $p(Chi^2)$: 0.881; $F(1,4) = 7.474$; $p(F) = 0.052$ for immobility. Based on $p(F) > 0.05$ no statistically significant concentration-response was found, and the slope is not significantly different from zero.

The obtained EC_{10} and EC_{20} values for the effect of Glyphosate acid on reproduction, immobility, and length of *Daphnia magna* are presented in the table below.

Table B.9.2.5.1-8: Re-calculated EC_{10} and EC_{20} values based on nominal concentrations

Endpoint (21 days)	Glyphosate acid technical [mg a.e./L]	
	Cumulative offspring per survived parent	Immobility
EC_{10} (95% CI)	56 – 100	n.d.
EC_{20} (95% CI)		n.d.

* = due to insufficient dose-response relationship true values could be deviating from calculated values
CI = confidence interval
n.d. = not detected

III. CONCLUSION

Assessment and conclusion by applicant:

The EC_{50} for parental immobility and reproduction were both calculated to be > 100 mg a.e./L (nominal). The overall no observed effect concentration (NOEC) was 56 mg a.e./L based on nominal concentrations.

All validity criteria according to the current OECD 211 were fulfilled .The study is therefore considered to be valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10} and EC_{20}, was performed (Position Paper No. CA 8.2.5.1/010) to fulfil the data requirements according to regulation EU 283/2013.

Re-calculated EC_{10} and EC_{20} values based on nominal test concentrations:

Endpoint (21 days)	Glyphosate acid technical [mg a.e./L]	
	Cumulative offspring per survived parent	Immobility
EC_{10} (95% CI)	56-100	n.d.
EC_{20} (95% CI)		n.d.

* = due to insufficient dose-response relationship true values could be deviating from calculated values
CI = confidence interval
n.d. = not determined

Thus, the NOEC of 56 mg a.e./L for immobility and reproduction is the most reliable and relevant endpoint to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

This study is considered valid.

The study was performed according to OECD 202, Part II. RMS reassessed the relevance of this study using the criteria of the current guideline OECD 211.

According to the current guideline OECD 211, the pH should be within the range 6 – 9. The pH at the highest concentration (pH 5.2-5.7 at 100 mg/L) was below this range. This deviation was

negatively correlated with increasing concentrations indicating that this pH decrease was due to the test item (intrinsic biochemical characteristic of glyphosate acid). Nevertheless, it is the opinion of the RMS that this pH remains acceptable for daphnids and that the effects observed may not be necessarily a consequence of glyphosate-induced acidification of the test medium. Although there might be an effect of pH at the highest concentration, the observed difference is substantial.

Since daphnids were held individually in the test vessel, it is possible to determine the exact number of offspring per parent and therefore a statistical evaluation according to the criteria of OECD 211 was made in this study. Based on this, a NOEC for reproduction was set at 56 mg a.s./L based on nominal concentration.

21-day NOEC for the reproduction of *Daphnia magna* exposed to glyphosate acid = 56 mg a.s./L (nominal).
EC10 values are not reliable for risk assessment purpose.

Data point:	CA 8.2.5.1/003
Report author	[redacted]
Report year	1993
Report title	21-day Reproduction Test in Daphnia Test Article: Glyphosate isopropylamine salt
Report No	80-91-2328-05-93
Document No	-
Guidelines followed in study	OECD Guideline 202, Part I and II.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary

The effects of glyphosate isopropylamine salt on reproduction of *Daphnia magna* were evaluated in a semi-static test. Prior to the inhibition and reproduction test, a preliminary acute toxicity test was performed to determine the concentration rage for the reproduction test.

For the definite reproduction test the following concentrations were tested: 43, 94, 207, 455 and 1000 mg test item/L, equivalent to 26.49, 57.90, 127.51, 280.28 and 616.0 mg glyphosate isopropylamine salt/L or 19.63, 42.90, 94.48, 207.68 and 456.43 mg glyphosate/L, respectively. In addition, a control group was exposed to synthetic test medium only.

Daphnids were observed for immobilisation and reproduction on day 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21. The adult daphnids were observed and the young counted and removed from the test vessels. Temperature, pH-value and oxygen saturation of the test solutions were measured at the test beginning and end each renewal period.

At the highest concentration level of 1000 mg/L, all specimens were found to be immobile on day 7. At or below a concentration of 207 mg/L, no significant immobilisation was observed. Reproduction was significantly inhibited at or above a concentration of 207 mg/L.

For the number of offspring, significant reductions in reproduction rate were observed at or above a concentration level of 207 mg/L, whereas at or below a concentration of 94 mg/L, significant increases were generally observed. However, on day 19, the reproduction rate was significantly reduced at a concentration of 455 mg test item/L. Therefore, it is considered more appropriate to determine the NOEC on the basis of the average number of off-spring per adult and day over the entire reproduction period. The 21-day EC_{50} for immobilisation was 587 mg test item/L, equivalent to 361.59 mg glyphosate isopropylamine salt/L or 267.93 mg a.e./L (nominal). The NOEC for immobilization was 207 mg test item/L, equivalent to 127.51 mg glyphosate isopropylamine salt/L and 94.48 mg a.e./L (nominal), respectively. The NOEC for reproduction rate was calculated to be 94 mg test item/L equivalent to 57.90 mg glyphosate isopropylamine salt/L and 42.90 mg a.e./L (nominal), respectively. All validity criteria according to the current OECD 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine salt
Description:	viscous liquid
Lot/Batch #:	01/06/93
Purity:	61.6% Glyphosate isopropylamine salt
Density:	1.23 g/cm^3 at 20°C

2. Test organism:

Species:	*Daphnia magna* Strauss
Age:	neonates (< 24 h old)
Size:	Not stated
Loading:	50 mL for each animal (reproduction test)
Source:	in-house laboratory breeding
Diet/Food:	Unicellular green algae (*Scenedesmus spp.)*
Acclimation period:	Daphnids were held in groups of ca.30 organisms in 1000 mL glass at standard test conditions. They were fed once daily on green algae.

3. Environmental conditions:

Temperature:	18 – 22°C
Photoperiod:	16 hours light / 8 hours dark, ~1000 lux
pH:	7.5 – 8.5
Dissolved oxygen:	> 60% of air saturation (approx. 6.0 mg O_2/L)
Conductivity:	0.049 µS/cm
Hardness:	14.5° dH

4. Experimental dates: August 27, 1993 to September 17, 1993

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The test was performed under semi-static conditions. Specimens were exposed to 43, 94, 207, 455 and 1000 mg test item/L, corresponding to 26.49, 57.90, 127.51, 280.28 and 616.0 mg glyphosate isopropylamine salt/L and 19.63, 42.90, 94.48, 207.68 and 456.43 mg glyphosate/L. In addition, a control group was exposed to the synthetic test media only. Stock solutions were prepared three times per week in which the solution was diluted with test water in a geometrical series by a factor or 2.2. Defined volumes of the stock solution were placed in a volumetric flask and filled up to the final volume of 2000 mL with synthetic test water (Elendt media). There were 8 vessels per treatment containing 5 daphnids each (500 mL glass beakers containing 50 mL test medium).

2. Observations: Daphnids were observed for immobilisation and reproduction on day 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21. The adult daphnids were observed and the young counted and removed from the test vessels. The adult daphnids were then transferred with specially prepared Pasteur pipettes. First, the young were filtered through a glass filter with 200 µL polypropylene mesh. Subsequently, the young were counted and the number of live and dead daphnids was noted. Three times a week the test medium was renewed. Subsequently, the offspring were counted and the number of live and dead animals was recorded. Temperature, pH-value and oxygen saturation were measured in line with each renewal period.

Analytical measurements were performed by HPLC analysis. Representative concentration levels of 43, 207, 455 and 1000 mg test item/L were analysed. The freshly prepared test medium was analysed on day 0, 5, 10, 14 and 19. As on day 7, no specimen survived at the highest concentration, analytical measurements were conducted on concentration levels 43, 207 and 455 mg test item/L.

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: The 21 d EC_{50} value was calculated according to Spearman and Karber.

Fecundity was analysed using a Man-Whitney-U-test (2-tailled, corrected for ties).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The average recovery of glyphosate in test media over 21 days was 87.5% and 93.7%, 98.7 and 99.6% of the nominal concentrations for 43, 207, 455 and 1000 mg test item/L, respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal in both tests, ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.5.1-9: Analytical results

	[mg/L]				
Nominal concentration of test item	**Control**	**43**	**207**	**455**	**1000**
Nominal concentration of glyphosate	**Control**	**19**	**94**	**207.67**	**456.43**
Mean measured value of Glyphosate over 21-day study	-	17.18	88.54	204.93	454.71
% of nominal	-	87.5	93.7	98.7	99.6

The NOEC value is given below are based on nominal concentrations.

Table B.9.2.5.1-10: Endpoints

Endpoints (21-day)	Test item [mg/L]
EC_{50} Immobilisation	587
NOEC Immobilisation	207
NOEC Reproduction	94

B. OBSERVATIONS

Observations: At the highest concentration level of 1000 mg/L, all specimens were found to be immobile on day 7. At or below a concentration of 207, no relevant immobilisation was observed. Results of the reproduction rate revealed significant inhibitory effects at or above a concentration of 207 mg/L.
For the number of off-spring, significant reduction in reproduction rate was observed mostly at or above a concentration level of 207 mg/L, whereas at or below a concentration of 94 mg/L, significant increases were generally observed. However, the reproduction rate was significantly reduced for all concentrations on day 19. The NOEC on the basis was determined of the average number of off-spring per adult and day over the entire reproduction period. Also, all validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

The percentage immobilisation is given below based on nominal concentrations.

Table B.9.2.5.1-11: Chronic toxicity of glyphosate isopropylamine salt to *Daphnia magna*

		Nominal concentration of test item [mg/L]				
Parameter	**Control**	**43**	**94**	**207**	**455**	**1000**
Immobilisation of adults after 21 d [%]	0.0	7.5	0.0	2.5	10.0	100
Total number of live off-spring from day 7 to day 21	5452	4941	5111	4426	3738	0
Mean number offspring per day per adult from day 7 to day 21	8.78	8.24	7.89	7.02*	6.05*	n.d.

* = statistically significant when compared to control (U-test according to Mann-Whitney), α = 0.05
n.d. = not determined

The results of the statistical analysis for ECx calculations are reported in the position paper summarised below. Applicant conclusion followed by assessment of RMS are presented after this position paper.

Data point	CA 8.2.5.1/011
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 80-91-2328-05-93 on the chronic toxicity of Glyphosate Isopropylamine salt to *Daphnia magna* under static-renewal conditions
Report No	110054-014
Document No	-
Guidelines followed in study	OECD Guideline No 202 (1984)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number:	80-91-2328-05-93
Author:	[redacted]
Substance:	Glyphosate isopropylamine salt
Title:	21 d Reproduction test in Daphnia test article: "Glyphosate isopropylamine salt"
Completion date:	24-11-1993
Test guideline(s):	OECD 202, Part II Reproduction Test (1984)
GLP:	Yes
Testing facility:	IBR Forschungs GmbH, Hannover, Germany
Sponsor:	Feinchemie Schwebda GmbH, Köln, Germany

B. STUDY DESIGN

Dates of work: August 2020

The study 80-91-2328-05-93 ([redacted] 1993) was statistically evaluated for the effects of glyphosate isopropylamine salt on the organism *Daphnia magna.* The organisms were exposed for 21 days to the following concentrations of glyphosate isopropylamine salt 43, 94, 207, 455, and 1000 mg IPA-salt /L (nominal). Considering a conversion factor of 0.741 31.9, 69,7, 153, 337, and 741 mg glyphosate acid equivalent/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:

In order to derive Effect Concentrations that have 10 and 20% effects on reproduction, expressed as cumulative offspring per introduced parent, probit analysis using linear maximum likelihood regression was used.

Cumulative offspring per survived parent was assessed by Weibul analysis using linear maximum likelihood regression.

For immobility, a logit analysis with linear maximum likelihood regression was used.

All statistical evaluations were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

For reproduction, expressed as cumulative offspring per introduced parent after 21 days, a decrease in reproduction was observed with increasing test concentration. The highest test concentration of 1000 mg/L resulted in a 100% reduction of reproduction compared to the control. The lowest test concentration 43 mg/L had with 9.4% a slightly higher reduction in reproduction than the next highest test concentration 94 mg/L with 6.9%.
The reproduction expressed as cumulative offspring per survived parent showed a clear dose-response relationship with increasing reduction of offspring with increasing test concentration. The highest test concentration 1000 mg/L resulted in a 100% reduction of reproduction.

For cumulative offspring per introduced parent, the parameters of the probit model are estimated as slope b: 7.68036, intercept a: - 220.88783.

Statistical parameters for goodness of fit are $Chi^2(38) = 3.297$; $p(Chi^2)$: 1.000; $F(1,38) = 6.6.03$, p(f) 0.014. A statistically significant concentration-response was found for offspring per introduced parent.

For cumulative offspring per survived parent, the parameters of the Weibul model are estimated as slope b: 7.32338; Intercept a: -20.72738.

Statistical parameters for goodness of fit are $Chi^2(3) = 0.02571$; $p(Chi^2)$: 0.999; $F(1,3) = 7.379$, p(f) 0.073. Based on $p = 0.5$, no significant concentration-response was found. However, based on visual observation of the data, dose-response is present (at $p = 0.073$). The model was used to derive an EC_{10} and EC_{20} with corresponding 95% confidence interval.

For immobility, the lowest test concentration 43 mg/L already resulted in 7.5% immobility, while the next highest test concentration 94 mg/L did not show any immobility at all. Thereafter, the percent immobility increased with increasing concentration. At the highest test concentration 1000 mg/L all daphnids were immobile.

The parameters for the logit model are estimated as slope b: 6.44220; Intercept a: -17.84339. Statistical parameters for goodness fit are: $Chi^2(3) = 353.63402$; $p(Chi^2)$: <0.001; $F(1,3) = 0.346$, p(F) = 0.598; the EC_{10} and EC_{20} for immobility calculations should therefore not be considered valid. The EC_{10} and EC_{20} values are estimated to be in the range of 455 to 1000 mg IPAsalt/L

The obtained EC_{10} and EC_{20} values for the effect of Glyphosate acid on reproduction, immobility, and length of *Daphnia magna* are presented in the table below.

Table B.9.2.5.1-12: Re-calculated EC_{10} and EC_{20} values based on nominal concentrations

Endpoint (21 days)	Glyphosate isopropylamine salt [mg/L]		
	Cumulative offspring per introduced parent	Cumulative offspring per survived parent	Immobility
EC_{10} (95% CI)	356 (155 – 407)	333 (171 – 649)	455 - 1000
EC_{20} (95% CI)	407 (223 – 447)	422 (264 – 675)	
	Glyphosate [mg a.e./L]		
EC_{10} (95% CI)	264 (115 – 302)	247 (127 – 481)	337 - 741
EC_{20} (95% CI)	302 (165 – 331)	313 (196 – 500)	

* = due to insufficient dose-response relationship true values could be deviating from calculated values
CI = confidence interval
n.d. = not determined

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of glyphosate isopropylamine salt on *Daphnia magna* were evaluated. The 21-day EC_{50} for immobilisation was 587 mg test item/L, corresponding to 361.59 mg a.s./L and 267.93 mg a.e./L (nominal). The NOEC for immobilization was 207 mg test item/L, equivalent to 127.51 mg a.s./L and 94.48 mg a.e./L (nominal), respectively. The NOEC for reproduction rate was calculated to be 94 mg test item/L equivalent to 57.90 mg a.s./L and 42.90 mg a.e./L (nominal), respectively.

All validity criteria according to the current OECD 211 were fulfilled. The study is considered to be valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10} and EC_{20}, was performed (Position Paper No. CA 8.2.5.1/011) to fulfil the data requirements according to regulation EU 283/2013.

Re-calculated EC_{10} and EC_{20} values based on nominal test concentrations:

Endpoint (21 days)	Glyphosate isopropylamine salt [mg/L]		
	Cumulative offspring per introduced parent	Cumulative offspring per survived parent	Immobility
EC_{10} (95% CI)	356 (155 – 407)	333 (171 – 649)	455 - 1000
EC_{20} (95% CI)	407 (223 – 447)	422 (264 – 675)	
	Glyphosate [mg a.e./L]		
EC_{10} (95% CI)	264 (115 – 302)	247 (127 – 481)	337 - 741
EC_{20} (95% CI)	302 (165 – 331)	313 (196 – 500)	

CI = confidence interval

Thus, the NOEC for immobilization at 127.51 mg/L and 94.48 mg a.e./L (nominal) and the NOEC for reproduction at 57.90 mg/L and 42.90 mg a.e./L (nominal) are the most reliable and relevant endpoints to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

This study is considered valid.

The study was performed according to OECD 202, Part II. RMS reassessed the relevance of this study using the criteria of the current guideline OECD 211.

40 animals were used at each concentration (8 groups of 5 animals). This is as recommended in initial version (1984) of the guideline. In the current version, for semi-static tests, (at least) 10 animals individually held at each test concentration are recommended. This improvement in the current guideline aims at determining the total number living offspring produced at the end of the test per parent daphnia at the start of the test excluding from the analysis parental accidental and/or inadvertent mortality. As no mortality was observed at the NOEC concentration (94 mg test item/L) and in control, RMS considers that the deviation has no impact on the outcome of this study.

According to the current guideline OECD 211, the pH should be within the range 6 – 9. The pH at the highest concentration (pH 5.58 at 1000 mg/L) was below this range. This deviation was negatively correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of test item). Nevertheless, it is the opinion of the RMS that this pH remains acceptable for daphnids and that the effects observed may not be necessarily a consequence of the induced acidification of the test medium.

EC10 values are available. However, given the dose-response and the 95% confidence interval of the ECx values, RMS agreed with the applicant that the NOEC is the most suitable endpoint for risk assessment.
NOEC for reproduction = 57.90 mg glyphosate isopropylamine salt/L and 42.90 mg glyphosate acid/L (nominal).

Data point:	CA 8.2.5.1/004
Report author	[redacted]
Report year	1990
Report title	Influence of glyphosate on the reproduction of *Daphnia magna*
Report No	250795
Document No	-
Guidelines followed in study	OECD 202, Part II, Reproduction Test (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary
The lethal and sub-lethal effects of glyphosate on *Daphnia magna* were evaluated in a 21-day toxicity test performed under semi-static conditions. The study was started with two glass beakers per test concentration, each containing 200 mL test solution. Two replicates of 10 *Daphnia* per concentration were exposed to 3.0, 9.4, 30, 94.9, and 300 mg a.s./L nominal concentrations. In addition, 2 x 10 *Daphnia* were exposed to test medium without test substance (blank control). After 7 days of exposure, 10 daphnids per test concentration and control with eggs in the brood pouch were selected and placed individually in a 100 mL beaker which contained 50 mL test solution. *Daphnia* were fed a mixture of yeast and algae (*Scenedesmus subspicatus*) at each test solution renewal.

Mortality of parent *Daphnia* and observation for the presence of alive and dead offspring were recorded

three times a week at the renewal of the test media.

The pH and dissolved oxygen concentration of the test samples were measured for all treatment periods at the beginning and end of the respective periods. The temperature was measured at the renewal of the test solutions.

The concentration of glyphosate in the test solutions was determined at the first and at the last treatment period (last water renewal) directly after treatment and at the end of the respective period in the 3.0, 30, and 300 mg a.s./L test vessels.

The mean measured concentrations of glyphosate in the test solutions ranged from 82.3 to 130.1% of nominal values. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results.
The authors concluded that the NOEC for survival and reproduction was 30 mg a.s./L based on nominal concentrations.
Given the dose response observed on reproduction data, RMS considered that the EC10 of 22.65 mg glyphosate acid./L based on nominal concentrations is a most suitable endpoint.
All validity criteria according to the OECD guideline 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Lot/Batch #:	198-SI-22-1
Purity:	98.7%

2. Vehicle of test material/media: Test medium

3. Test organism:

Species:	*Daphnia magna*
Age of animals:	Neonates (< 24 h old)
Loading:	First 7 days of exposure: 10 *Daphnia* in 200 mL test solution; Form day 7 to day 21: 1 *Daphnia* in 50 mL test solution
Source of organisms:	Continuous laboratory cultures

4. Environmental conditions:

Temperature:	21.5-22.5°C
pH:	5.2-8.3 (new solutions) 5.3-8.5 (old solutions)
Dissolved oxygen:	8.6-8.8 mg O_2/L (new solutions) 8.3-8.7 mg O_2/L (old solutions)
Conductivity:	Not mentioned in the report
Hardness:	Not mentioned in the report
Photoperiod:	16 hours light /8 hours dark; 500-2000 lux

5. Experimental dates: January 17, 1990 to February 07, 1990

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The lethal and sub-lethal effects of glyphosate on *Daphnia magna* were

evaluated in a 21-day toxicity test performed under semi-static conditions. The study was started in two glass beakers per test concentration, each containing 200 mL test solution. Two replicates of 10 *Daphnia* per concentration were exposed to 3.0, 9.4, 30, 94.9, and 300 mg a.s./L nominal concentrations. In addition, 2 x 10 *Daphnia* were exposed to test medium without test substance (blank control). After 7 days of exposure, 10 daphnids per test concentration and control with eggs in the brood pouch were selected and placed individually in a 100 mL beaker which contained 50 mL test solution. *Daphnia* were fed a mixture of yeast and algae (*Scenedesmus subspicatus*) at each test solution renewal.

A stock solution of 500 mg a.s./L was prepared on day 0 by dissolving 500 mg test item in 1000 mL of test medium. This solution was freshly prepared on days 2, 5, 7, 9, 12, 14, 16, and 19 of the exposure period. Appropriate amounts of this stock solution were diluted to prepare the test concentrations.

2. Observations: Mortality of P_0 generation of *Daphnia* and observation for the presence of alive and dead offspring were recorded three times a week at the renewal of the test media. Dead P_0 *Daphnia* and offspring were removed at the observation dates.

The pH and dissolved oxygen concentration of the test samples (controls, the lowest (3.0 mg a.s./L) and the highest (300 mg a.s./L) test concentrations of glyphosate) was measured at all treatment periods at the beginning and at the end of the respective periods. The temperature was measured at the renewal of the test solutions. The concentration of glyphosate in the test solutions was determined at the first and at the last treatment period (last water renewal) directly after treatment and at the end of the respective period in the 3.0, 30, and 300 mg a.s./L test vessels.

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Steel-Test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate in the test solutions ranged from 82.3 to 130.1% of the nominal values for the 3.0, 30, and 300 mg a.s./L test concentrations. On the basis of the analytical data, the nominal concentrations were used for the calculation and reporting of all results.

Table B.9.2.5.1-13: Analytical results

	[mg glyphosate/L]					
Nominal concentration	**Control**	**3.0**	**9.4**	**30.0**	**94.9**	**300**
Day 0 mean concentration	-	2.821	-	27.71	-	390.4
Day 2 mean concentration	-	3.183	-	31.40	-	365.8
Day 19 mean concentration	-	2.585	-	27.08	-	
Day 21 mean concentration	-	3.404	-	29.63	-	
Mean measured over 21 day study	-	2.99	-	28.95	-	378.1
% of nominal over 21d	-	99.9	-	96.5	-	126

study						

The endpoint value is given below.

Table B.9.2.5.1-14: Endpoints

Endpoints	[mg a.s./L]
21-day NOEC for survival and reproduction	30 mg/L

B. OBSERVATIONS

Reproduction of young daphnids started on day 9 of the exposure period. No statistically significant influence of glyphosate on the reproduction rate was observed up to a concentration of 30 mg a.s./L. At the highest tested concentration of 300 mg a.s./L all daphnids were dead after 5 days of exposure.
The effects of glyphosate on *Daphnia magna* mortality and reproduction are shown below.

Table B.9.2.5.1-15: Effects of glyphosate on *Daphnia magna* mortality and reproduction

Nominal concentration [mg a.s./L]	Mean adult mortality [%]	Total number of off-spring per parent animal	Total off-spring
Control	0	127±24	1266
3.0	0	123±29	1226
9.4	0	134±22	1338
30	0	102±26	1023
94.9	10	48±29*	476
300	100	0	0

* Statistically significant difference

All validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

The results of the statistical analysis for ECx calculations are reported in the position paper summarised below. Applicant conclusion followed by assessment of RMS are presented after this position paper.

Data point	CA 8.2.5.1/012
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 250795 on the chronic toxicity of Glyphosate to *Daphnia magna* under static-renewal conditions
Report No	110054-015
Document No	-
Guidelines followed in study	OECD Guideline No 202 (1984) and, EPA Guidelines 540/9-82-024, 72-4
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: 250795
Author: [redacted]
Substance: Glyphosate
Title: Influence of Glyphosate on the reproduction of Daphnia magna
Completion date: 25-09-1990
Test guideline(s): OECD 202, Part II Reproduction Test (1984), EPA Guidelines 540/9-82-024, 72-4
GLP: Yes
Testing facility: RCC Umweltchemie AG, Itingen, Switzerland
Sponsor: A/S CHEMINOVA, Lemvig, Denmark

B. STUDY DESIGN

Dates of work: August 2020

The study 250795 ([redacted] 1990) was statistically evaluated for the effects of glyphosate on the organism *Daphnia magna.* The organisms were exposed for 21 days to the following concentrations of glyphosate; 3, 9.4, 30, 94.9, and 300 mg test item/L (nominal). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:

In order to derive Effect Concentrations that have 10 and 20% effects on reproduction, expressed as cumulative offspring per introduced parent and cumulative offspring per survived parent, of the test subjects (EC_{10} and EC_{20}), a 3-parametric logistic CDF analysis with Levenberg-Marquardt optimization using non-linear regression was performed. Confidence limits were estimated by Monte-Carlo simulation using the parameter errors obtained from the inverse Hessian matrix.

For survival, a Weibull analysis with linear maximum likelihood regression was used.

All statistical evaluations were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

For reproduction, expressed as cumulative offspring per introduced parent after 21 days, a decrease in reproduction was observed with increasing test concentration, except for the second highest test concentration 9.4 mg/L, which showed a slight increase in reproduction. The highest test concentration of 300 mg/L resulted in a 100% reduction of reproduction compared to the control.
The reproduction expressed as cumulative offspring per survived parent showed the same pattern.

For cumulative offspring per introduced parent, the parameters of the 3 parameter logistic CDF model are estimated as b0: 127.54, b1: 68.556, b2: 1.984.
For cumulative offspring per survived parent, the parameters of the 3 parameter logistic CDF model are estimated as b0: 12.740, b1: 70.721, b2: 1.959.

According to the statistical parameters; F (2, 3) = 118.658; p(F) = <0.001; R^2 = 0.761 for cumulative offspring per introduced parent, and F (2, 3) = 114.315; p(F) = <0.001; R^2 = 0.753 for cumulative offspring per survived parent, calculations of the EC_{10} and EC_{20} values should be considered valid.

After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.392 for cumulative offspring per introduced parent and 0.342 for cumulative offspring per survived parent.

The calculation of EC_{10} and EC_{20} values was possible for these parameters.

For survival, at the second highest test concentration 94 mg/L a mortality of 10% was observed. At the highest test concentration 300 mg/L all daphnids were dead. The test was started at d 0 with 10 daphnids per beaker (2 replicates), and at day 7 the daphnids were transferred and the test was continued with 1 daphnid per beaker (10 replicates). Although the highest test concentration of 300 mg/L already showed 90% mortality at day 2, the number of starting daphnids at day 0 was set to 10 for the statistical assessment of the data.

The parameters for the Weibull model are estimated as slope b: 9.09181; Intercept a: -20.22886. Statistical parameters for goodness fit are: $Chi^2(3)$ = 0.01029; p(Chi^2) = 1.000; F(1,3) = 1027.659, p(F) <0.001; the EC_{10} and EC_{20} for immobility calculations should therefore be considered valid. Since p(F) was <0.05 the slope of the relationship is significantly different from zero. However, the calculation of EC_{10} and EC_{20} values was possible for this parameter.

The obtained EC_{10} and EC_{20} values for the effect of Glyphosate acid on reproduction, immobility, and length of *Daphnia magna* are presented in the table below.

Table B.9.2.5.1-16: Re-calculated EC_{10} and EC_{20} values based on nominal concentrations

Endpoint (21 days)	Glyphosate [mg a.s./L]		
	Cumulative offspring per introduced parent	Cumulative offspring per survived parent	Immobility
EC_{10} (95% CI)	22.65 (9.98 - 33.51)	23.04 (9.78 - 34.46)	94.94 (n.d.)
EC_{20} (95% CI)	34.08 (19.76 -45.94)	34.86 (19.79 - 47.37)	114.81 (n.d.)

CI = confidence interval
n.d. = not determined

III. CONCLUSIONS

Assessment and conclusion by applicant:

Lethal and sub-lethal effects of glyphosate on *Daphnia magna* were evaluated in a 21-day toxicity test. The 21-day NOEC for survival and reproduction of *D. magna* exposed to glyphosate was 30 mg a.e./L based on nominal concentrations. All validity criteria according to the current OECD 211 were fulfilled in the test.
The study is considered to be valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10} and EC_{20} was performed (Position Paper No. CA 8.2.5.1/012) to fulfil the data requirements according to regulation EU 283/2013.

Re-calculated EC_{10} and EC_{20} values based on nominal test concentrations:

Endpoint (21 days)	Glyphosate [mg a.s./L]		
	Cumulative offspring per introduced parent	Cumulative offspring per survived parent	Immobility
EC_{10} (95% CI)	22.65 (9.98 - 33.51)	23.04 (9.78 - 34.46)	94.94 (n.d.)
EC_{20} (95% CI)	34.08 (19.76 -45.94)	34.86 (19.79 - 47.37)	114.81 (n.d.)

CI = confidence interval
n.d. = not determined

Thus, the NOEC of 30 mg a.s./L for survival and reproduction is the most reliable and relevant endpoint to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

This study is considered valid.

The study was performed according to OECD 202, Part II. RMS reassessed the reliability of this study using the criteria of the current guideline OECD 211.

Experiment was started with two replicates of 10 daphnia per concentration (20 individuals per concentration). This is less than recommended in initial version (1984) of the guideline (40 individuals per concentration). Thereafter, in this study, the ten daphnia (individually held) were selected at day 7. In the current version of the guideline, for semi-static tests, (at least) 10 animals individually held at each test concentration are recommended. This improvement in the current guideline aims at determining the total number living offspring produced at the end of the test per parent daphnia at the start of the test excluding from the analysis parental accidental and/or inadvertent mortality. Then RMS considers that the reproduction rate was adequately assessed and this deviation (to the current protocol) is deemed acceptable.

According to the current guideline OECD 211, the pH should be within the range 6 – 9. The pH at the highest concentration (pH 5.2 at 300 mg/L) was below this range. This deviation was negatively

correlated with increasing concentrations indicating that this was due to the test item (intrinsic biochemical characteristic of test item). Nevertheless, it is the opinion of the RMS that this pH remains acceptable for daphnids and that the effects observed may not be necessarily a consequence of the induced acidification of the test medium.

RMS notes that the total number of off-spring per parent animal at the proposed NOEC (30 mg/L) is of only 80% of control. The decrease also appears to be dose-dependant (97%, 100%, 80%, 38% and 0% at 3.0, 9.4, 30, 94.4 and 300 mg/L respectively).
RMS considers that the most suitable endpoint for risk assessment should be based on the EC10 of 22.65 mg a.e./L (95% CI: 9.98 - 33.51).

Data point:	CA 8.2.5.1/005
Report author	[redacted]
Report year	1989
Report title	21-Day Prolonged Static Renewal Toxicity of Glyphosate Technical to *Daphnia magna*
Report No	AB 89-58
Document No	-
Guidelines followed in study	OECD Guideline 202 U.S. Guideline 72-4, (EPA-FIFRA, 40 CFR, Section 158.145).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary

The effects of glyphosate on the reproduction of *Daphnia magna* were evaluated in a 21-day semi-static test. The test was performed using nominal concentrations of 6.5, 13, 25, 50 and 100 mg test item/L. In addition, a control group was exposed to dilution water. The test solutions were prepared using hard blended water. The test solutions were renewed three times a week. There were four glass jars per treatment, each containing ten daphnids.

Samples for analytical confirmation were taken initially and at each renewal. Recoveries were ranging from 92.3, to 108.0% of nominal concentrations. Therefore, ecotoxicological endpoints were based on nominal concentrations of the test item.

Starting at test initiation, observations were made daily, recording the number of immobile *Daphnia magna*. Furthermore, behavioural or sublethal effects as well as any gross pathogenic or toxic response were recorded. Furthermore, survival, abnormal effects and time to first brood of daphnids were recorded daily throughout the study. Reproduction success was measured by counting and discarding the offspring produced in each concentration 3 days a week for the duration of the study.

No effects of glyphosate technical on survival, reproduction and time to first brood of *Daphnia magna* after 21-day exposure were observed in any test item treatment. No effects on behaviour were observed

for the duration of the study. EC_{50} was determined to be > 100 mg a.e./L. The NOEC was determined to be 100 mg test item/L. All validity criteria according to OECD 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XLI-203
Purity:	97.67%

2. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 specimens in 400 mL test solution
Source:	In-house culture
Diet/Food:	Once daily with a suspension of *Selenastrum capricornutum* (8×10^7 cells/400 mL), supplemented with a Tetramin®, cereal leaves and yeast suspension
Acclimation period:	None

3. Environmental conditions:

Temperature:	20 ± 2°C
Photoperiod:	16 hours light / 8 hours dark (approx. 431 – 861 Lux), with 30-minute dawn and dusk transition periods
pH:	6.8 – 8.2 (new solutions), 7.4 – 7.9 (old solutions)
Dissolved oxygen:	New solutions: 8.3 – 9.0 mg/L (89.5 to 101% saturation) Old solutions (2-3 days after renewal) : 4.1 – 6.8 mg/L (47 to 80% saturation)
Conductivity:	350 µS/cm
Hardness:	174 mg $CaCO_3$/L.

4. Experimental dates: April 4, 1989 to April 25, 1989

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity of glyphosate on *Daphnia magna* was evaluated in a 21-days prolonged semi-static test, using nominal concentrations of 6.5, 13, 25, 50 and 100 mg test item/L. In addition, a control group was exposed to dilution water. The test solutions were prepared using hard blended water prepared to a total hardness of between 160 and 180 mg $CaCO_3$/L. The test solutions were renewed three times a week. There were four glass jars per treatment, each containing ten daphnids (1000 mL glass jars containing 400 mL test medium).

2. Observations: Observations were made on a daily basis to record the number of immobile *Daphnia magna*, starting from test initiation. Furthermore, behavioural or sublethal effects as well as any gross pathogenic or toxic response were recorded. Any dead individuals were immediately removed from the testing solutions. In addition, survival, effects on behaviour and observance of first brood of the organisms were recorded daily throughout the study. Reproduction success was measured by counting and discarding the offspring produced in each concentration three times a week for the duration of the

study. Temperature, pH-value and oxygen saturation of the test solutions were measured on solution renewal days. In addition, total hardness and specific conductivity of the dilution water was measured weekly. Samples for analytical confirmation of the new solutions were taken initially and at each renewal days. The analytical data are reported separately (Monsanto Study No. ML-89-62).

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: The test parameters of survival, time to first brood (days), and total young/adult reproduction were analysed using analysis of variance. Dunnett's Test was used for mean separation. The 21-day EC_{50} values were calculated by probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements (separate report ML-89-62) were performed to determine the concentration of glyphosate in test solutions. Result showed recoveries of 92.3, 100%, 108.0%, 100% and 100% for nominal concentrations of 6.5, 13, 25, 50 and 100 mg test item/L. Therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

The 21-day EC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.5.1-17: Endpoints

Endpoints (21-day)	**Glyphosate [mg a.e./L]**
EC_{50} 21-day (95% C.I.)	> 100
NOEC 21-day	≥ 100

B. OBSERVATIONS

No effects of glyphosate technical on survival, reproduction and time to first brood of *Daphnia magna* were observed after 21-days of exposure in all test item concentrations. No effects on behaviour of adults and offspring were observed during the course of the study.

Table B.9.2.5.1-18: Lethal effects of glyphosate to *Daphnia magna* (mean values)

Glyphosate [mg a.e./L]	**Control**	**6.5**	**13**	**25**	**50**	**100**
Survival (21-day) [%]	98	100	100	100	100	98
Reproduction (21-day) (young adult/reproduction day) (± SD)	5.2 ± 0.2	5.1 ± 0.3	5.3 ± 0.2	5.1 ± 0.1	5.1 ± 0.2	5.1 ± 0.0
Mean number of young adult/adult (21-days)	73.7	72.7	74.2	71.4	72.7	71.0
Time to first brood (days) (± SD)	7.8 ± 0.5	7.8 ± 0.5	8.0 ± 0.0	8.0 ± 0.0	7.8 ± 0.5	8.0 ± 0.0

All validity criteria according to the current guideline OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a 21-day prolonged semi-static reproduction study with *Daphnia magna*, no effects of glyphosate technical on survival, reproduction, and time to first brood of *Daphnia magna* were observed. Therefore, the 21-day EC_{50} was determined to be > 100 mg a.e./L (nominal). The NOEC was determined to be ≥ 100 mg a.e./L (nominal). All validity criteria according to the current guideline OECD 211 were fulfilled. The study is considered to be valid and reliable for the regulatory risk assessment for glyphosate.

A statistical evaluation concluded that re-calculation of EC_{10} and EC_{20} was not possible due to lack of effects both in the reproduction and immobility of the daphnids in this study. Thus, the NOEC of 100 mg a.e./L (nominal) is the most reliable and relevant endpoint to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

The study was performed according to OECD 202, Part II. RMS reassessed the relevance of this study using the criteria of the current guideline OECD 211.

40 animals were used at each concentration (4 replicates of 10 animals). This is as recommended in initial version (1984) of the guideline. In the current version, for semi-static tests, (at least) 10 animals individually held at each test concentration are recommended. This improvement in the current guideline aims at determining the total number living offspring produced at the end of the test per parent daphnia at the start of the test excluding from the analysis parental accidental and/or inadvertent mortality. As only one dead daphnid was observed at the NOEC concentration (100 mg test item/L) and in control (none died at intermediate concentrations), RMS considers that the deviation has no impact on the outcome of this study.

Analytical control measurements of the actual concentrations of the test item were performed and the results are reported in a separate study (study number: ML-89-62). The study ML-89-62 was checked by RMS and the recovery values reported in the study summary are agreed. Nominal concentrations can be used to set endpoint.
This study is considered valid. No ECx values could be derived due to the lack of effects.

NOEC = 100 mg glyphosate acid/L (nominal)

Data point:	CA 8.2.5.1/006
Report author	[redacted]
Report year	1982
Report title	Chronic Toxicity of Glyphosate to *Daphnia magna* Under Flow-Through Test Conditions
Report No	AB 82-036
Document No	-
Guidelines followed in study	ASTM Committee, (Draft No. 5, September,1979, E-35.2; Draft No. 3, 1981, E-47.01; Draft No. 2, September,1979, E-35.21)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS):	Valid

Executive Summary

The effects of glyphosate on the reproduction of *Daphnia magna* were evaluated in a 21-day chronic test in flow-through conditions. The test was performed using nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L. In addition, a control group was exposed to untreated water. The test solutions were permanently renewed using a one-litre proportional diluter system. There were four replicates per treatment, each containing 10 test daphnids.

The number of immobile *Daphnia magna* was recorded three times a week. Furthermore, reproductive success was measured by recording the number of off-spring produced in each treatment on every observation day for the duration of the study. In addition to survival and reproduction data, growth of adult daphnids was determined at the termination of the test.

No significant decrease in survival or length of adult daphnids was observed in organisms exposed to glyphosate for 21 days. Length of daphnids in the lowest (26 mg/L) and highest (365 mg/L) glyphosate treatment groups was significantly greater than in the control.

Reproduction significantly decreased at the three highest test item concentrations (96, 186 and 365 mg glyphosate/L). In contrast to that, at the lowest test item concentration (26 mg/L) an increase of reproduction when compared to the control was observed.

The author concluded that the NOEC was 50 mg test item/L (nominal).

RMS considered that the results should be expressed as mean measured concentrations as some measured values are below 80% of nominal. The 21d-NOEC (reproduction) *Daphnia magna* was 41 mg glyphosate acid/L (mean measured concentration).

All validity criteria according to the OECD guideline 211 were fulfilled. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate standard
Description:	White powder
Lot/Batch #:	NBP 1782610 [1992049]
Purity:	99.7%

2. Vehicle of test material/media: Deionized water

3. Test organism:

Species:	*Daphnia magna*
Age:	Neonates (< 24 h old)
Loading:	10 specimens for 1000 mL test solution
Source:	In-house culture
Diet/Food:	Once daily with *Pseudokirchneriella subcapitata*
Acclimation period:	None

4. Environmental conditions:

Temperature:	20 ± 2°C
Photoperiod:	16 hours light / 8 hours dark (approx. 538 – 753 Lux)
pH:	8.1 – 8.2 (control), 6.1 – 6.2 (highest test concentration)
Dissolved oxygen:	7.0 – 9.0 mg/L
Conductivity:	50 µS/cm
Hardness:	255 mg $CaCO_3$/L.

5. Experimental dates: March 5, 1982 to March 26, 1982

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxic effects of glyphosate on *Daphnia magna* were evaluated in a 21-day flow-through test, using nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L. In addition, a control group was exposed to untreated water. The test solutions were prepared using well water at ABC's Aquatic Bioassay Laboratory, with known characteristics (hardness = 255 mg $CaCO_3$/L, pH = 8.2). The test system consisted of six sets of 1 L quadruplicate chambers, which were immersed in a circulating water bath. The test solutions were permanently renewed using a one-litre proportional diluter system, with modifications to allow intermittent delivery of large stock volumes of glyphosate and dilution water into the test chambers. The renewal rate was 200 mL/aquarium every 120 minutes, an amount sufficient to replace the 1 L test volume 3 times in a 24-hour period. There were four replicates per treatment, each containing 10 test daphnids, which were randomly placed in test chambers.

2. Observations: Observations were made three times a week (every Monday, Wednesday and Friday) to record the number of immobile *Daphnia magna*, starting from test initiation. Furthermore, the reproductive success was measured by recording and discarding the offspring produced in each concentration on every observation day for the duration of the study. Growth of adult daphnids was determined at test termination.

Temperature, pH-value and oxygen saturation of the test solutions were measured on day 0, 4, 7, 14 and 21 in control, and nominal test item treatments of 25, 99 and 397 mg glyphosate/L.

Samples for analytical confirmation of the concentration of glyphosate in test solutions were taken and analysed.

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Measured parameters in the quadruplicate test chambers were analysed using one-way analyses of variance (ANOVA) and as a post hoc test, Fisher's Protected Least Significant Difference (LSD), was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements were performed to determine the concentration of glyphosate in test solutions. The mean measured concentrations of glyphosate in test solutions were 26, 50, 96, 186 and 378 mg glyphosate/L for the nominal concentrations of 25, 50, 99, 199 and 397 mg test item/L respectively. Analytical recovery ranged from 93 to 104% of nominal concentrations.

Table B.9.2.5.1-1: Analytical results

		mg glyphosate/L				
Nominal concentrations	Control	25	50	99	199	397
Day 0 measured concentrations	-	17	32	61	115	250
Day 4 measured concentrations	-	25	44	90	175	356
Day 7 measured concentrations	-	22	44	82	155	306
Day 14 measured concentrations	-	24	43	83	162	332
Day 21 measured concentrations	-	21	42	79	157	312
Mean measured concentrations over study period	-	26	50	96	186	378

The NOEC value is given below are based on nominal concentrations.

Table B.9.2.5.1-2: Endpoints

Endpoints (21-day)	**Glyphosate [mg a.e./L]**
NOEC 21-day	50

B. OBSERVATIONS

No significant decreases in survival or length of adult daphnia were observed in all test item treatments. Length of daphnids in the lowest (26 mg/L) and highest (378 mg/L) glyphosate concentrations were significantly greater than control.

Reproduction significantly decreased at the three highest test item concentrations (96, 186 and 365 mg glyphosate/L). At the lowest level of glyphosate (27 mg/L) an increase of reproduction when compared to controls was observed. The highest test item concentration not resulting in decreased reproduction was 50 mg/L. An increase of length and reproduction of daphnids observed at the lowest test item concentration is not considered to be deleterious and thus not used to estimate the NOEC.

Table B.9.2.5.1-3: Adult length, survival and young produced per adult reproductive day of *Daphnia magna* continuously exposed to glyphosate during a 21-day life cycle study

Glyphosate [mg a.e./L] (mean measured concentrations)	**Control**	**26**	**50**	**96**	**186**	**378**
		21.8#	**41.0#**	**79.0#**	**152.8#**	**311.2#**
Survival (21-day) [%]	100	98	100	98	98	98
Reproduction (21-day) (young adult/reproduction day) (± SD)	4.9 ± 0.42	6.5 ± 0.15 *	5.1 ± 0.49	4.1 ± 0.78 *	3.8 ± 0.10 *	1.7 ± 0.32 *
Adult length (mm) (± SD)	3.7 ± 0.06	3.9 ± 0.05 *	3.7 ± 0.07	3.6 ± 0.11	3.7 ± 0.10	3.8 ± 0.03 *
Mean number of young adult/adult (21-days)	68.6	91.5	70.5	55.7	52.5	23.5

recalculated by RMS based on observed concentrations (see commenting box for details)
* Significantly different (Fishers' LSD, $\alpha = 0.05$).

All validity criteria according to the current OECD 211 were fulfilled, as immobility of daphnids in control groups was <20% and the mean number of live off-spring produced per parent animal surviving at the end of test was ≥60.

The results of the statistical analysis for ECx calculations are reported in the position paper summarised below. Applicant conclusion followed by assessment of RMS are presented after this position paper.

Data point	CA 8.2.5.1/013
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study AB 82-036 on the chronic toxicity of Glyphosate to *Daphnia magna* under flow-through conditions
Report No	110054-016
Document No	-
Guidelines followed in study	ASTM Committee E-35.21. 4p., Draft No. 5 (1979), ASTM Committee E47.01.31 p., Draft No. 3 (1981)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: AB 82-036
Author: [redacted]

Substance:	Glyphosate
Title:	Chronic toxicity of Glyphosate to Daphnia magna under flow-through test conditions
Completion date:	09-09-1982
Test guideline(s):	ASTM Committee E-35.21. 4p., Draft No. 5 (1979), ASTM Committee E47.01.31 p., Draft No. 3 (1981)
GLP:	Yes
Testing facility:	Analytical Biochemistry Laboratories, Columbia, Missouri, USA
Sponsor:	Monsanto Chemical Company, St. Louis, Missouri, USA

B. STUDY DESIGN

Dates of work: August 2020

The study AB 82-036 ([redacted] 1982) was statistically evaluated for the effects of glyphosate on the organism *Daphnia magna*. The organisms were exposed for 21 days to the following concentrations of glyphosate; 25, 50, 99, 199, and 397 mg test item/L (nominal). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive Effect Concentrations that have 10 and 20% effects on reproduction, expressed as offspring per adult per reproductive day of the test subjects (EC_{10} and EC_{20}), a Weibull analysis using linear maximum likelihood regression was performed.

For immobility all effects were below 10%. Therefore, no statistical analysis was performed.

All statistical evaluations were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

For reproduction, expressed as offspring per adult per reproductive day after a 21-day exposure to glyphosate, a decrease in reproduction was observed with increasing test concentration, starting at the third highest test concentration 99 mg/L. The highest test concentration of 397 mg/L resulted in 65% decrease of reproduction compared to the control, see table below.

The parameters for the Weibull model are estimated as slope b: 4.04824; Intercept a: -10.49640. Statistical parameters for goodness fit are: $Chi^2(3) = 0.00806$; $p(Chi^2)$: 1.000; $F(1,3) = 33.921$, $p(F) = 0.010$; $R^2 = 0.919$ the EC_{10} and EC_{20} for offspring per adult per reproductive day calculations should therefore be considered valid.

For immobility, no effects were observed. The immobility was not greater than 2.5% at any tested concentration compared to the control and therefore the calculation of EC_{10} and EC_{20} values was not possible for this parameter.

The obtained EC_{10} and EC_{20} values for the effect of Glyphosate acid on reproduction and immobility of *Daphnia magna* are presented in the table below.

Table B.9.2.5.1-42: Re-calculated EC_{10} and EC_{20} values based on nominal concentrations

Endpoint (21 days)	Glyphosate [mg a.e./L]	
	Offspring/adult/reproductive day	Immobility
EC_{10} (95% CI)	108.88 (58.81 - 201.59)	n.d.
EC_{20} (95% CI)	166.85 (111.24 - 250.25)	n.d.

* = due to insufficient dose-response relationship true values could be deviating from calculated values
CI = confidence interval
n.d. = not determined

III. CONCLUSIONS

Assessment and conclusion by applicant:

In a 21-day chronic toxicity study, the exposure of *Daphnia magna* to glyphosate resulted in reduced reproduction at or above the nominal concentration of 99 mg a.e./L. No other adverse compound-related effects were observed. The NOEC was determined to be 50 mg a.e./L (nominal). All validity criteria according to the current OECD 211 were fulfilled.

The study is considered to be valid and reliable for the regulatory risk assessment for glyphosate.

A statistical re-evaluation addressing EC_{10} and EC_{20} was performed (Position Paper No. CA 8.2.5.1/013) to fulfil the data requirements according to regulation EU 283/2013.

Re-calculated EC_{10} and EC_{20} values based on nominal test concentrations:

Endpoint (21 days)	Glyphosate [mg a.e./L]	
	Offspring/adult/reproductive day	Immobility
EC_{10} (95% CI)	108.88 (58.81 - 201.59)	n.d.
EC_{20} (95% CI)	166.85 (111.24 - 250.25)	n.d.

CI = confidence interval
n.d. = not determined

Thus, the NOEC of 50 mg a.e./L for reproduction and the NOEC of 100 mg a.e./L for survival are the most reliable and relevant endpoints to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

This study was not conducted under GLP (GLP was not compulsory at the time the study was performed).

The study was performed according to OECD 202, Part II. RMS reassessed the relevance of this study using the criteria of the current guideline OECD 211.

40 animals were used at each concentration (4 replicates of 10 animals). This is as recommended in initial version (1984) of the guideline OECD 202. In the current version (OECD 211), for semi-static tests, (at least) 10 animals individually held at each test concentration are recommended. This improvement in the current guideline aims at determining the total number living offspring produced at the end of the test per parent daphnia at the start of the test excluding from the analysis parental

accidental and/or inadvertent mortality. As no dead daphnid was observed at the proposed NOEC concentration (50 mg test item/L) and in control, RMS considers that the deviation has no impact on the outcome of this study.

Analytical control measurements of the actual concentrations of the test item were performed. The available information is reported below.

TABLE 2: Measured concentrations of Glyphosate during the 21 day chronic life cycle toxicity bioassay with Daphnia magna.

Nominal Residue Levels (mg/l)	Measured Concentration (mg/l)										
	Day 0		Day 4		Day 7[a]		Day 14[a]		Day 21[a]		Correcte
	Observed	Corrected	Observed	Corrected	Observed	Corrected	Observed	Corrected	Observed	Corrected	Mean (±S
Control	0.14		0.14		0.13		0.16		0.08		
Level 1 (25)	17	23	25	29	22	27	24	28	21	25	26 (±2.
Level 2 (50)	32	44	44	52	44	53	43	50	42	51	50 (±3.
Level 3 (99)	61	84	90	106	82	99	83	97	79	95	96 (±8.
Level 4 (199)	115	158	175	206	155	187	162	188	157	189	186 (±17
Level 5 (397)	250	342	356	419	306	369	332	386	312	376	378 (±28
+2.5 ppm Spike	1.70 (68%)		2.03 (81%)		20.16 (81%)		22.80 (81%)		19.64 (79%)		
+10 ppm Spike	7.08 (71%)		8.70 (87%)		82.80 (83%)		82.80 (83%)		79.64 (80%)		
+40 ppm Spike	31.7 (79%)		34.2 (86%)		341 (85%)		334 (84%)		361 (90%)		

[a]Spikes on these days were 25, 100 and 400 ppm rather than 2.5, 10 and 40 ppm.

The method has been considered fit for purpose (see Volume 3 B.5). There is no need to correct the analysed concentration. Thus RMS recalculated the mean measured concentrations based on observed values. The table below presented the calculation. Considering that some measured values are below 80% of nominal, RMS considered that the results should be expressed as mean measured concentrations.

Nominal conc. mg/L	Analysed concentrations in mg/L (% of nominal)					Mean meas. conc. mg/L (% of nominal)
	Day 0	Day 4	Day 7	Day 14	Day 21	
25	17 (68)	25 (100)	22 (88)	24 (96)	21 (84)	21.8 (87%)
50	32 (64)	44 (88)	44 (88)	43 (86)	42 (84)	41.0 (82%)
99	61 (62)	90 (91)	82 (91)	83 (84)	79 (80)	79.0 (80%)
199	115 (58)	175 (88)	155 (88)	162 (81)	157 (79)	152.8 (77%)
397	250 (63)	356 (90)	306 (90)	332 (84)	312 (79)	311.2 (78%)

EC10 can be calculated for reproduction only (not for immobility). The applicant considered nominal concentrations in ECx calculations. Given that some measured concentrations were found below or close to 80%, RMS considered that the results should be expressed as mean measured concentrations. ECx values were therefore not retained.

21d-NOEC (reproduction) *Daphnia magna* = 41 mg glyphosate acid/L (mean measured concentration).

Data point:	CA 8.2.5.1/007
Report author	[redacted]
Report year	2011
Report title	AMPA (Aminomethylphosphonic acid): A semi-static life cycle toxicity test with the Cladoceran (*Daphnia magna*)
Report No	139A-393
Document No	-
Guidelines followed in study	OECD Guideline 211 (1998), ASTM E 1193-97
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 211 (2012):* *Minor:* • *Survival in the negative control group was slightly below the 80%* *This does not affect the reliability of the study.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary

The effects of AMPA (aminomethylphosphonic acid) on the survival, growth and reproduction of *Daphnia magna* were evaluated in a 21-day reproduction test under semi-static conditions with renewal of test medium every 2 to 3 days. The reproduction test was performed using a geometric series five nominal test concentrations (7.5, 15, 30, 60 and 120 mg AMPA/L) and a dilution water control (negative control). 10 replicates with one daphnid each were prepared per test concentration and 20 replicates with one daphnid each for the control.

Parental *Daphnia magna* were observed on a daily basis for mortality, onset of reproduction and signs of toxicity. Body length and dry weights of surviving parental specimens were measured at the end of the exposure period. The number of juvenile daphnia produced in each vessel was counted three times per week and at test termination. Mean measured test concentrations were determined from samples of test media collected from each treatment and control group at test initiation, at the end of the first renewal cycle, at the beginning and end of the longest renewal cycle during the second week of the test, and at the beginning and end of the last renewal cycle (test termination).

AMPA was not detected in the control group. The mean measured concentrations of AMPA in samples collected during the test for each treatment group were 7.4, 15, 30, 57 and 120 mg AMPA/L, equivalent to 99, 100, 100, 95 and 100% of the nominal concentrations, respectively. Therefore, the results evaluation is based on nominal test concentrations. There was no significant mortality observed during the test when compared to the control. Treatment related effects on growth were observed at 60 and 120 mg AMPA/L. There was significant decrease in mean neonate production observed in the 30, 60 and 120 mg AMPA/L treatment groups.

Survival in the negative control group was slightly below the 80% validity criterion required in the OECD 211 test guideline. However, this minor deviation is not considered to have had a significant impact on the validity of this study as the surviving daphnids in the control replicates appeared normal and healthy throughout the test suggesting that the mortality observed was most likely attributable to incidental death and not related to the health of the organisms.

Adult daphnids in the control group produced an average of 227 live young per surviving adult (CV = 11.6%), which is well above the validity criterion of ≥60 live young per surviving adult. Therefore, the study is considered valid according to OECD 211.

The overall no observed effect concentration (NOEC) based on reproduction (juvenile production) was determined to be 15 mg AMPA/L. The study is considered to be valid.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	Solid
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7%

2. Vehicle of test material/media: ASTM medium

3. Test organism:

Species:	*Daphnia magna* Straus
Age:	Neonates (< 24 h old)
Loading:	1 daphnid per 200 mL test medium
Source:	In-house culture
Diet/Food:	Daily; mixture of yeast, cereal grass media and trout chow (YCT) and suspension of *Pseudokirchneriella subcapitata*

4. Environmental conditions:

Temperature:	19.0 – 20.8 °C
Photoperiod:	16 hours light Light intensity = 314 lux
pH:	7.1 – 8.6
Dissolved oxygen:	6.8 – 9.1 mg O_2/L
Conductivity:	274 – 391 µS/cm
Hardness:	132 - 140 mg $CaCO_3$/L

5. Experimental dates: February 09, 2011 to March 04, 2011

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A 21-day reproductive toxicity test was conducted under semi-static conditions, with renewal of test medium every 2 to 3 days. *Daphnia magna* neonates (<24 hours old) were exposed to nominal concentrations of 7.5, 15, 30, 60 and 120 mg AMPA/L in moderately hard dilution water (ASTM medium). In addition, a negative control group was prepared in parallel. Ten glass vessels (250 mL vessels containing 200 mL test medium each) were used per treatment group for the test item and 20 vessels for the control group. One daphnid (neonate < 24 hours old) was exposed per replicate (vessel).

2. Observations: The number of living, immobilised and dead parental *Daphnia magna* and the time to gravidity (presence of eggs in brood pouch) were observed on a daily basis. Body length and dry weights of surviving parental specimens were measured at the end of the exposure period (21 days).

The number of neonate daphnids was counted three days a week and their condition was recorded. The presence of unhatched eggs was recorded, when observed. Incidental mortality was also recorded, when occurred. At the end of the test, body length and dry weight of each surviving parental daphnid was measured.

The temperature, pH-values and the oxygen saturation were measured at test initiation, before and after the renewal of the test media in two replicate test chambers and at test termination. Hardness, alkalinity and specific conductance were measured in batch solutions of the negative (dilution water only) control and at the highest test item concentration at test initiation and on one renewal day each week and from pooled replicate solutions at test termination.

Analytical measurements were performed by using an HPLC method of analysis using samples taken from all test concentrations for the freshly prepared solutions, at the end of the first renewal cycle (old solution), and at the beginning and end of last renewal cycle. For the aged test media, samples were taken from 2 alternate replicates of each treatment and control group and pooled by treatment group.

The validity criteria according to the current OECD 211 guideline are the following:

- In the control, the mortality of the parent animals (female Daphnia) should not exceed 20% at the end of the test.
- In the control, the mean number of living offspring produced per parent animal surviving at the end of the test should be > 60.

3. Statistical calculations: Data were statistically tested using Chi-square and Fisher's Exact test (discrete-variable data; $\alpha = 0.05$) and Dunnett's t-test (one-tailed, normal distributed data; $\alpha = 0.05$). The NOEC was determined by visual interpretation of the results.

II. RESULTS AND DISCUSSION

A. FINDINGS

Concentrations of AMPA in the freshly prepared test solutions, sampled on Days 0, 9 and 19 ranged from 92.5 to 106% of the nominal concentrations. Concentrations of AMPA in the old test solutions sampled immediately prior to renewal on Days 2, 12 and at test termination on Day 21 ranged from 78.6 to 117% of the nominal concentrations. The overall mean measured concentrations of AMPA during the test were 7.4, 15, 30, 57 and 120 mg AMPA/L, equivalent to 99, 100, 100, 95 and 100% of the nominal concentrations, respectively. Since the mean measured test concentrations were within the 80 – 120% of nominal test concentration, the results of the study are reported as nominal test concentrations.

Table B.9.2.5.1-53: Analytical results

	[mg AMPA/L]					
Nominal concentration	**Control**	**7.5**	**15**	**30**	**60**	**120**
Day 0 mean concentration (fresh)	-	7.41	15.8	30.9	62.7	127
Day 2 mean concentration	-	6.21	12.8	24.5	47.2	97.9
Day 9 mean concentration (fresh)	-	7.05	13.9	29.3	56.3	112
Day 12 mean concentration	-	7.90	15.9	35.2	58.5	137
Day 19 mean concentration (fresh)		7.64	14.0	28.0	55.7	114
Day 21 mean concentration		8.04	15.7	32.4	61.4	133
Mean measured over 21-day study	-	7.4	15	30	57	120
% of nominal over 21d study	-	99	100	100	95	100

The 21-day EC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.5.1-64: Endpoints

Endpoints	AMPA [mg/L]
EC_{50} (21 days) for parental survival and immobility	> 120
NOEC (21 days) for parental survival and immobility	120
EC_{50} (21 days) for reproduction (95% C.I.)	90 (84 – 94)
NOEC (21 days) for reproduction	15
EC_{50} (21 days) for growth (95% C.I.)	90 (84 – 94)
NOEC (21 days) for growth	30
Overall LOEC	30
Overall NOEC	15

B. OBSERVATIONS

Survival in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups at test termination was 80, 100, 70, 100 and 90%, respectively. No significant differences were detected in any treatment group in comparison to the control ($\alpha = 0.05$, Fisher's Exact test). In the 120 mg AMPA/L treatment group, all surviving parental daphnids appeared pale and smaller in comparison to the control organisms from Day 5 through test end.

The first day of brood production in the controls and in all AMPA treatments indicated no delay in the onset of egg production at any of the AMPA concentrations tested. No aborted or shed eggs were present in the control or in any of the AMPA treatments. No males or ephippia were observed during the test.

Adult daphnids in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups produced an average of 229, 213, 189, 169 and 59.6 live young per surviving adult, respectively. Dunnett's test indicated there was a statistically significant decrease in mean neonate production in the 30, 60 and 120 mg AMPA/L

treatment groups (30, 57 and 120 mg AMPA/L as mean measured concentration) in comparison to the negative control (α = 0.05).

In the control group , the mean body length was 5.3 mm and mean dry weight was 0.99 mg. Daphnids in the 7.5, 15, 30, 60 and 120 mg AMPA/L treatment groups had mean lengths of 5.2, 5.2, 5.1, 5.3 and 4.3 mm, respectively, and mean dry weights of 0.99, 1.0, 0.97, 0.69 and 0.45 mg, respectively. Dunnett's test indicated a significant decrease in length in the 30 and 120 mg AMPA/L (30 and 120 mg AMPA/L as mean measured concentration) treatment groups in comparison to the negative control (α = 0.05).

However, the decreases noted in the 30 mg AMPA/L treatment group was not dose related. Dunnett's test indicated there was a statistically significant decrease in dry weight in the 60 and 120 mg AMPA/L (57 and 120 mg AMPA/L as mean measured concentration) treatment groups in comparison to the control (α = 0.05).

Table B.9.2.5.1-75: Chronic toxicity of AMPA to *Daphnia magna*

	Control	**AMPA [mg/L]**				
		7.5	**15**	**30**	**60**	**120**
Mortality of adults after 21 d [%]	25	20	0	30	0	10
Mean number offspring per adult	227±26.3	229 ±24.8	213 ±26.6	189 ±19.7*	169 ±22.1*	59.6 ±13.4*
Mean length of offspring	5.3 ±0.14	5.2 ±0.16	5.2 ±0.12	5.1 ±0.16*	5.3 ±0.18	4.3 ±0.17*
Mean dry weight of offspring	0.99 ±0.24	0.99 ±0.12	1.0 ±0.22	0.97 ±0.25	0.69 ±0.20*	0.45 ±0.15*

* Indicates a statistically significant decrease in comparison to the negative control (Dunnett's one-tailed test, α = 0.05).

After 21 days of exposure, survival in control group was 75%. Although survival in the negative control group was slightly below the 80% criterion in OECD 211, this small difference is not considered to have impacted the validity of this study. The surviving daphnids in the control replicates appeared normal and healthy through until test end indicating that the mortality observed was attributed to incidental death and not the health of the organisms. Adult daphnids in the control group produced an average of 227 live young per surviving adult (CV = 11.6%), well above the validity criterion of ≥60 live young per surviving adult. Therefore, the study is considered valid according to OECD 211.

The results of the statistical analysis for ECx calculations are reported in the position paper summarised below. Applicant conclusion followed by assessment of RMS are presented after this position paper.

Data point	CA 8.2.5.1/014
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 139A-393 on the chronic toxicity of Aminomethylphosphonic acid to *Daphnia magna* under static-renewal conditions
Report No	110054-017
Document No	-
Guidelines followed in study	OECD Guideline No. 211 (2008), ASTM Standard E 1193-97 (1997)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: 139A-393
Author: [redacted]
Substance: Aminomethylphosphonic acid (AMPA)
Title: AMPA (Aminomethylphosphonic acid): A semi-static life-cycle toxicity test with the cladoceran (*Daphnia magna*)
Completion date: 17-06-2011
Test guideline(s): OECD 211 (2008), ASTM Standard E 1193-97 (1997)
GLP: Yes
Testing facility: Wildlife International, Ltd., Easton, Maryland, USA
Sponsor: Monsanto Company (on behalf of the Glyphosate Task Force)

B. STUDY DESIGN

Dates of work: August 2020

The study 139A-393 ([redacted] 2011) was statistically evaluated for the effects of Aminomethylphosphonic acid (AMPA) on the organism *Daphnia magna*. The organisms were exposed for 21 days to the following concentrations of AMPA; 7.5, 15, 30, 60, and 120 mg test item/L (nominal). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:

In order to derive Effect Concentrations that have 10 and 20% effects on reproduction, expressed as cumulative offspring per survived parent, and length of the test subjects (EC_{10} and EC_{20}), a 3-parametric normal CDF analysis with Levenberg-Marquardt optimization using non-linear regression was performed. For immobility, a logit analysis using linear maximum likelihood regression was performed.

All statistical evaluations were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

For reproduction, expressed as cumulative offspring per survived parent after 21 days, a decrease in reproduction was observed with increasing test concentration, starting at the second lowest test concentration 15 mg/L with a 6% reduction in reproduction compared to the control. The highest test concentration of 120 mg/L resulted in a 74% reduction of reproduction compared to the control.

For cumulative offspring per survived parent, the parameters of the 3-parametric normal CDF model are estimated as b0: 220.994, b1: 1.591, b2: 0.268. According to the statistical parameters; F (2, 3) = 147.374; p(F) = <0.001; R^2 = 0.818 for cumulative offspring per survived parent, calculations of the EC_{10} and EC_{20} values should be considered valid.

After non-linear regression a lack of fit was detected for the function (p(F|Lack of Fit) = 0.015 for cumulative offspring per survived parent and other statistical models did not result in a better fit. However, the calculation of EC_{10} and EC_{20} values was possible for this parameter.

For immobility, no effects were observed. The control showed the highest percentage of immobile daphnids with 25%. At the highest test concentration of 120 mg/L, 10% of the daphnids were immobile, see table below.

The parameters for the logit model are estimated as slope b: -0.92697; Intercept a: -1.14443. Statistical parameters for goodness fit are: $Chi^2(3)$ = 3.02591; p(Chi^2) = 0.388; F(1,3) = 0.520, p(F) = 0.523; the EC_{10} and EC_{20} for immobility calculations should therefore be considered valid. Since p(F) > 0.05, no statistically significant dose-response relationship was found and the calculation of EC_{10} and EC_{20} values was not possible for this parameter.

For length, no clear dose-response relationship was observed. The second highest test concentration 60 mg/L resulted in the smallest decrease in length of 0.6%, while already the lowest test concentration 7.5 mg/L gave a 2.4% decrease in length. The highest test concentration 120 mg/L showed a 18.9% in length compared to the control.

For length, the parameters of the 3-parametric normal CDF model are estimated as b0: 5.222, b1: 2.063, b2: 0.046. According to the statistical parameters; F (2, 3) = 118.982; p(F) = <0.001; R^2 = 0.803 for length, calculations of the EC_{10} and EC_{20} values should be considered valid.

After non-linear regression a lack of fit was detected for the function (p(F|Lack of Fit) = 0.015 for length and other statistical models did not result in a better fit. However, the calculation of EC_{10} and EC_{20} values was possible for this parameter.

The obtained EC_{10} and EC_{20} values for the effect of Glyphosate acid on reproduction and immobility of *Daphnia magna* are presented in the table below.

Table B.9.2.5.1-86: Re-calculated EC_{10} and EC_{20} values based on nominal concentrations

Endpoint (21 days)	AMPA [mg/L]		
	Cumulative offspring per survived parent	Immobility	Length
EC_{10} (95% CI)	38.99 (30.51 - 49.83)	n.d.	115.64 (n.d.)
EC_{20} (95% CI)	51.15 (40.05 - 65.55)	n.d.	> 120

CI = confidence interval
n.d. = not determined

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of AMPA (aminomethylphosphonic acid) on the survival, growth and reproduction of *Daphnia magna* were evaluated in a 21-day reproduction test.

The nominal based EC_{50} values for reproduction, immobility and growth were 90 mg/L, ≥ 120 mg/L and 90 mg/L, respectively.

The no observed effect concentrations (NOEC) for immobility and growth were 30mg/L and ≥ 120 mg/L, respectively. The NOEC based on reproduction was determined to be 15 mg/L (nominal) for AMPA exposed daphnids.

The study is considered to be valid.

A statistical re-evaluation addressing EC_{10} and EC_{20} was performed (Position Paper No. CA 8.2.5.1/014) to fulfil the data requirements according to regulation EU 283/2013.

Re-calculated EC_{10} and EC_{20} values based on nominal test concentrations:

Endpoint (21 days)	AMPA [mg/L]		
	Cumulative offspring per survived parent	Immobility	Length
EC_{10} (95% CI)	38.99 (30.51 - 49.83)	n.d.	115.64 (n.d.)
EC_{20} (95% CI)	51.15 (40.05 - 65.55)	n.d.	> 120

CI = confidence interval
n.d. = not determined

Thus, the NOEC for immobility and growth were 30 mg/L and 120 mg/L, respectively. The NOEC based on reproduction was determined to be 15 mg/L. These are the most reliable and relevant endpoints to be considered in the regulatory risk assessment.

Assessment and conclusion by RMS:

The validity criteria of OECD 211 on mortality in the control group was not fulfilled as it should not exceed 20 % at the end of the test. The authors of the study attributed the slight difference on mortality compared to validity criteria to incidental death as the surviving daphnids in the control replicates appeared normal and healthy throughout the test. Moreover an average of 227 live young per surviving adult was produced in the control which is well above the validity criterion of ≥ 60 live young per surviving adult. Thus, this deviation is not considered to have had a significant impact on the validity of this study. RMS considers the study valid and reliable for risk assessment.

The pH was within the recommended range 6 – 9. The effects observed are therefore not a consequence of the induced acidification of the test medium.

EC_{10} values for cumulative offspring per survived parent at 21 days and length are 38.99 mg/L and 115.64 mg/L, respectively.
EC_{20} values for cumulative offspring per survived parent at 21 days and length are 51.15 mg/ and > 120 mg/L, respectively.
No EC_{10} and EC_{20} values could be calculated for immobility as no effects were observed.

Overall NOEC based on reproduction = 15 mg AMPA/L (nominal).

Data point:	CA 8.2.5.1/008
Report author	Levine, S.L. *et al.*
Report year	2015
Report title	Aminomethylphosphonic acid has low chronic toxicity to *Daphnia magna* and *Pimephales promelas*
Document No	DOI: 10.1002/etc.2940 E-ISSN: 1552-8618
Guidelines followed in study	OECD 211 (2008), OECD 210 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from current OECD guideline 211 (2012): None*
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability (RMS):	Yes/Reliable The daphnid part of this publication actually corresponds to the study summarized above and already assessed by RMS (CA 8.2.5.1/007, [redacted] 2011, AMPA (Aminomethylphosphonic acid): A semi-static life cycle toxicity test with the Cladoceran (Daphnia magna), 139A-393). The fish part of this publication actually corresponds to the study summarized above and already assessed by RMS (CA 8.2.2.1/004, [redacted] 2011, AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (Pimephales promelas), 139A-39A).

For detailed summary of this article proposed as relevant and reliable by both applicant and RMS please refer to the appendix to Volume 3 CA B.9 on literature data related to ecotoxicology under point B.9.2.5.

B.9.2.5.2. Reproductive and development toxicity to an additional aquatic invertebrate species

B.9.2.5.2.1. Development and emergence in *Chironomus riparius*

Data point:	CA 8.2.5.3/001
Report author	[redacted]
Report year	2020
Report title	MON 77973: A Study on the Toxicity to the Sediment Dweller *Chironomus riparius* Using Spiked Water
Report No	20FV2ME
Document No	-
Guidelines followed in study	OECD guideline 219 (2004)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the guideline OECD 219 (2004):* *Minor:* - *Samples of sediment and pore water were not taken or analysed based on the concentrations of the test item in the overlying water measured during the range-finding test (>80% of nominal at test start in the overlying water column at start of exposure and > 50% of nominal for the duration of the range-finding trial). Analysis of overlying water only is therefore considered to be sufficient and to reflect the exposure situation in this study. An impact on the integrity of this study can therefore be excluded.* - *Several midges in the control emerged later than required in the guideline. Since total emergence in the control exceeded 90% of inserted animals, and since more than 89% of the emerged control midges had emerged by day 23, this is not considered to have any impact on the integrity of the study*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Supportive

Executive Summary

In a sediment-water toxicity test using spiked water first-instar larvae of freshwater dipteran *Chironomus riparius* were exposed to MON 77973 concentrations of 100 and 1000 mg a.e./L according to OECD 219 for 28 days. Exposure concentrations were based on results of a range-finding test conducted at 0.1 – 1000 mg a.e./L. The test was conducted using a limit test design at the two rates with eight replicates prepared per test item concentration and the control, with 20 organisms added per test vessel. Three times per week, the larvae were fed using a TetraMin® suspension, with the food ration increased accordingly during the test. At least three times per week the test vessels were observed in order to visually assess any behavioural differences compared with the control. Daily from day 11 the vessels were checked for emerged midges.

A concentration-response relationship of MON 77973 was not observed for emergence ratio and development rate after 28 days of exposure. A statistically significant inhibition compared to the control was not found up to and including the highest test concentration. Therefore, NOEC and LOEC values were 1000 mg a.e./L and > 1000 mg a.e./L, respectively, based on nominal test concentrations.

RMS considered this study as informative only. In addition, no report for analytical method was available (see Volume 3 CA B.5). This is not a validity criteria, thus the study is still supportive but the overall reliability (and weight in weight of evidence) is reduced.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON 77973 (Glyphosate acid)
Description:	White crystalline powder
Lot/Batch #:	11493988
Purity:	97.7 wt% (acid equivalent: a.e.)

2. Vehicle of test material/media: Test medium

3. Test organism:

Species:	*Chironomus riparius* (Meigen)
Age of animals:	1st instar larvae
Loading:	20 larvae/vessel
Fill volume:	570 mL
Replication:	8 replicates per test item concentration and the control
Source of animals:	House cultures, originally supplied by Aventis, D-65962 Frankfurt am Main
Diet/Food:	TetraMin® suspension three times a week Feed rate: - Day 0 – 10 = 0.25 – 0.5 mg TetraMin® per day - Day 11 until end = 0.5 – 1 mg TetraMin® per day
Acclimation period:	Egg masses and hatching larvae were maintained for at least 5 days prior to addition to the test vessels as 1st instar larvae. Animal addition occurred one day prior to spiking. Animal addition occurred after sediment had been added to test vessels and covered with test medium and acclimated under test conditions for 2 days.

4. Environmental conditions:

Artificial Sediment:	Yes, according to OECD 219 (2004), peat content 4.8% of sediment dry weight; sediment water ratio approx. 1:4
Temperature:	19.8-21.3°C
Photoperiod:	16 h light:8 h dark
pH range	7.6-8.3
Duration:	28 days
Dissolved oxygen range	7.1-9.1
Hardness:	254-336 mg/L $CaCO_3$

5. Dates of experimental work: 10th February to 24th March 2020

B. STUDY DESIGN AND METHODS

Experimental conditions
First-instar larvae were exposed to MON 77973 concentrations of 100 and 1000 mg a.e./L according to OECD 219 for 28 days. Eight replicates were used per test item concentration and the control designed as a limit test. Prior to application of the test item, the formulated sediment was conditioned for 7 days. For this purpose it was covered with Medium M4 (sediment:water volume ratio 1:4 (±≤ 0.5)) and was incubated under the same conditions which prevailed in the subsequent test.

A stock solution was prepared by adding 10.0 g nominal of the test item to 1000 mL of test medium. After 2 min ultrasonication and 30 min stirring, the test item had dissolved and the stock solution appeared clear. This stock solution was used undiluted as the application solution for preparation of treatment of 1000 mg/L. 100 mL of this stock solution were diluted to 1000 mL in order to prepare the application solution for treatment of 100 mg/L. The chironomid larvae were introduced into the test vessels one day prior to spiking. One day after addition of the larvae, the test item was added to the overlying water of each test vessel.
Per test vessel, an aliquot of 57 mL (nominal) of the application solutions were carefully mixed with the nominal volume of 513 mL of test medium present in each test vessel to obtain a total volume of 570 mL. Vessels were aerated daily on workdays in all test vessels. Three times per week, the larvae were fed with TetraMin®. The food ratio was 0.25–0.5 mg TetraMin® per day and larva from day 0 to day 10 and 0.5–1 mg TetraMin® per day and larva from day 11 until the end of the exposure. At least three times per week the test vessels were observed in order to assess visually any behavioural differences compared with the control. Daily from day 11, the vessels were checked for emerged midges. Dissolved oxygen content and pH were measured in one test vessel of each concentration level and the control at start of exposure and once per week; in all test vessels at the end of the exposure. Temperature was monitored in one test vessel of each concentration level and the control at start of exposure and once per week and in all test vessels at the end of the exposure.

Analytical procedures
To verify the nominally applied concentrations, samples were taken from the overlying water.

Statistical calculations
To determine whether there were sex-specific effects, a Chi^2-Contingency test (one-sided greater; alpha 0.05) was performed. Since there was no significant effect on the sex ratio, the biological parameters emergence ratio and development rate were evaluated for pooled male and female emerged midges. Dunnett's multiple t-test procedure was used to evaluate whether there were significant differences between the control and the various test item concentrations (emergence ratio and development rate). Normal-distribution of data was tested with the Kologorov-Smirnov test (alpha: 0.01). Levene's test (p: 0.01) was used to test variance homogeneity. In one of the replicates, zero midges emerged during the test. The reasons are not clear, since oxygen concentrations, aeration monitoring and observation of test vessels documentation gave no hint. This replicate was therefore excluded from statistical evaluation of emergence. The statistical software package ToxRatPro® 3.3.0 (ToxRat Solutions GmbH, Naheweg 15, D-52477 Alsdorf) was used for these calculations.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data:

Summary of analytical results: Concentrations of Glyphosate: acid equivalent (a.e.) measured in the overlying water.

Test period [d]	Nominal concentration (mg test item/L))	Nominal concentration (mg active ingredient/L))*	Measured concentration [mg active ingredient/L]	% of nominal concentration
0	Control	0	n.d.	n.a.
0	100	97.7	87.3	89.4
0	1000	977	798	81.7
28	Control	0	n.d.	n.a.
28	100	97.7	54.5	55.8
28	1000	977	695	71.1

* using the test item purity of 97.7 wt% (a.e.); Limit of Quantification (LOQ) was 10 mg/L for water; n.d.: not detectable (< Limit of detection: 3 mg/L for water); n.a.: not applicable.

The Chi2 r-x-2-Contingency test (alpha = 0.05) showed no significant effect on the sex ratio of the emerged midges. Therefore, the biological parameters emergence ratio (ER) and development rate (DR) were evaluated for pooled male and female emerged midges.

Table B.9.2.5.2.1-1: Number and emergence ratio of midges emerged per replicate of each treatment at end of exposure.

Nominal concentration [mg a.e./L]	**Number of midges emerged**								
Replicate	**a**	**b**	**c**	**d**	**E**	**f**	**g**	**h**	**Mean**
Control	18	17	19	18	18	19	19	17	18.1
100	17	17	17	17	18	20	18	18	17.8
1000	0	17	18	19	20	18	19	15	15.8
	Emergence ratio								
Control	0.90	0.85	0.95	0.90	0.90	0.95	0.95	0.85	0.906
100	0.85	0.85	0.85	0.85	0.90	1.00	0.90	0.90	0.888
1000	0.00	0.85	0.90	0.95	1.00	0.90	0.95	0.75	0.788

Table B.9.2.5.2.1-2: Mean development rates [1/d] of the midges (males & females, pooled) per replicate of each treatment and mean development rate per treatment.

Concentration [mg a.e./L]	**Control**	**100**	**1000**
Replicate			
a	0.04121	0.04916	-
b	0.05340	0.05645	0.05087
c	0.04850	0.05309	0.05002
d	0.04829	0.04557	0.04839
e	0.05157	0.05577	0.04557
f	0.05235	0.04871	0.05165
g	0.05067	0.04970	0.05188
h	0.05105	0.04944	0.04995
Mean	**0.04963**	**0.05099**	**0.04976**
SD	0.003826	0.003760	0.002193

B. OBSERVATIONS

No concentration-dependent observations were recorded. Across all treatments, emerged midges were occasionally observed to be unfit to fly or reluctant to leave the water surface. One dead pupa was observed at 100 mg a.e./L. A concentration-response relationship of MON 77973 was not observed for emergence ratio and development rate after 28 days of exposure. A statistically significant inhibition compared to the control was not found up to and including the highest test concentration. Therefore, NOEC and LOEC values were ≥ 1000 mg a.e./L dry sediment and > 1000 mg a.e./L, respectively.

Validity criteria

In order to consider the test to be valid according to OECD 219, the following conditions should be fulfilled:

- The emergence in the controls must be at least 70% at the end of the test (observed: 90.6%)
- Emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels (observed: between days 13 and 28*)
- At the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel. The oxygen concentration should be at least 60% of the air saturation value at the temperature used, and the pH of overlying water should be in the 6-9 range in all test vessels.

(measured: oxygen ≥90% ASV; pH of 7.8–8.3)

- the water temperature should not differ by more than ± 1.0 °C (measured: 19.8–21.3 °C)

*Several midges in the control emerged later than required in the guideline. However, since total emergence in the control exceeded 90% of inserted animals, and since more than 89% of the emerged control midges had emerged by day 23, this is not considered to have any impact on the integrity of the study. The study is therefore considered valid.

III. CONCLUSIONS

Assessment and conclusion by applicant:
In a sediment-water toxicity test using spiked water, *Chironomus riparius* was exposed to MON 77973 concentrations of 100 and 1000 mg a.s./L according to OECD 219. Based on nominal concentrations, the derived NOEC and LOEC were ≥ 1000 mg a.s./L and > 1000 mg a.s./L, respectively.
The study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:
New study.

The test item was added to the overlying water (spiked water), static test.
Two concentration levels were tested (extended limit test).

Deviations to validity criteria:
Several midges in the control emerged later than required in the guideline. However, since total emergence in the control exceeded 90% of inserted animals, and since more than 89% of the emerged control midges had emerged by day 23, study authors considered this had no impact on the integrity of the study. RMS agrees.

Analytical verification:
It is mentioned in the report that samples of sediment and pore water were not taken or analysed (as requested by the sponsor). According to the study author, this formal deviation from the test guideline is justified based on the concentrations of the test item in the overlying water measured during the range-finding test. In this rangefinding test, the nominal concentrations of the test item were substantially achieved (>80% of nominal) in the overlying water column at start of exposure

and maintained above 50% of nominal for the duration of the range-finding trial. Analysis of overlying water only was therefore considered to be sufficient by the study authors.
OECD 219 requires (besides overlying water) samples of the pore water and the sediment at the start (preferably one hour after application of test substance) and at the end of the test, at the highest concentration and a lower one.. Based on its fate characteristics, glyphosate is considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint.

pH:
The pH of the stock solution was very low (pH=2.00) and adjusted to 6.6 using 9.2 mL of 7-mol NaOH. The adjustment of pH may however mask the intrinsic biochemical characteristics of glyphosate acid. The magnitude of pH decrease in natural conditions should be considered in the risk assessment. It is RMS opinion that the acidity in laboratory test conditions is expected to far exceed the acidity in surface waters in natural conditions and it can be assumed that it can be reasonably assumed that pH decrease (if any) will not affect the midge larvae in natural conditions. This should nevertheless be considered in the risk assessment.

Sediment:
- peat content 4.8% of sediment dry weight
- sediment water ratio approx. 1:4
- Organic carbon: 2.5% of dry sediment

Dilution water: Elendt medium M4
According to the guideline OECD 219, if there is an interaction suspected between hardness ions and the test substance, lower hardness water should be used (and thus, Elendt Medium M4 must not be used in this situation). Total hardness in the overlying water was of 14.2-18.8 °dH / 254-336 mg CaCO3/L, $NH4^+$ of 6.13 mg/L. The use of Elendt Medium M4 artificial media was not justified. However, in long term daphnia tests (e.g. [redacted] 1995) the same medium did not have an impact on the concentration levels. Thus the media can be considered acceptable.

Other remarks:
In one replicate at 1000 mg test item/L, zero midges emerged during the test. The reasons are not clear, since oxygen concentrations, aeration monitoring and observation of test vessels documentation gave no hint. This replicate was therefore excluded from statistical evaluation of emergence. As it occurs in only one replicate out of 8, and that no effect were observed in the other 7 replicates, RMS considers this replicate A as an outlier.

Overall conclusion:
Chironomus riparius was exposed to MON 77973 concentrations of 100 and 1000 mg a.s./L, and no statistically significant effects have been observed up to the highest tested dose.
Based on its fate characteristics, glyphosate is considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint. However according to the current EFSA guidance[3] a test is not required on chironomus when the chronic Daphnia test (or other comparable study with e.g. Chironomus) does not show a EC10/NOEC of < 0.1 mg/L (even in case of partitioning of the substance in the sediment). Then this study is not required for the risk assessment. The absence of analytics in sediment do not allow deriving a robust endpoint, this study is considered informative only. In addition, no report for analytical

[3] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

method was available (see Volume 3 CA B.5). This is not a validity criteria, thus the study is still supportive but the overall reliability (and weight in weight of evidence) is reduced.

B.9.2.5.2.2. Sediment dwelling organisms

Based on its fate characteristics, glyphosate and AMPA are considered as persistant in sediment and chronic exposure of the sediment dwellers is expected. According to EU Reg 283/2013 section 8.2.5.4 test using spiked sediment or at least analytics in sediment is required to set an endpoint.
However according to the current EFSA guidance on aquatic organisms (2013) and the EFSA opinion on sediment organisms (2015), sediment toxicity studies are triggered when the water–sediment study indicates that > 10 % of the applied radioactivity is present in the sediment at or after day 14 and the outcome of a chronic Daphnia test (or another comparable study with insects) results in an EC10 (or NOEC) < 0.1 mg/L. Since the lowest chronic Daphnia endpoint is greater than 0.1 mg/L, this study is not considered necessary for risk assessment purpose.

However for compliance with the EU Reg 283/2013, further information to assess the effects of glyphosate and AMPA on sediment dwelling organisms is required (data gap).

In relation with e-fate data gap, further information to assess the risk assessment for metabolite 1-oxo-AMPA for sediment dwelling organisms is necessary (data gap). For details, please refer to Volume 3 CA B.8 point B.8.2.2.5.

B.9.2.6. Effects on algal growth

Studies on effects of the active substance glyphosate and its relevant metabolites on aquatic macrophytes to fulfil the data requirements according to EU Regulation No 283/2013 are presented in the following.

B.9.2.6.1. Effects on growth of green algae

Data point:	CA 8.2.6.1/001
Report author	[redacted]
Report year	2002
Report title	A study on the Toxicity of Glyphosate isopropylamine salt 62.5% to Algae (*Pseudokirchneriella subcapitata*)
Report No	A-99-02-04
Document No	-
Guidelines followed in study	OECD Guideline 201, EEC Directive 92/69 C.3
Deviations from current test guideline by the applicant: See RMS analysis in RMS comment box	*Deviation from the guideline OECD 201 (2011):* *Minor:* *- The pH-values of the algal medium recommended by, "Schlösser (1982). Sammlung von Algenkulturen, Pflanzenphysiologisches Institut der Universität Göttingen (SAG) - List of Strains", were lower than reported in OECD 201. In correlation with the slightly lower pH values measured in concentration 100.0 mg/L there could be an effect on the growth rate of the algae.* *- Analysis of the results were based on average recovery value instead of the geometric mean concentrations.* *The study is considered valid as all validity criteria were met.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of glyphosate isopropylamine salt on *Pseudokirchneriella subcapitata* were evaluated in a 96-hour static toxicity test at nominal concentrations of 4.27, 9.39, 20.66, 45.45 and 100 mg test item/L. A negative control group (culture medium only) was prepared in parallel. The test vessels were 300 mL Erlenmeyer glass flasks containing 100 mL of control or test medium .The initial algal cell concentration was 1 x 104 cells/mL. At 24, 48, 72 and 96 hours, the algal cell densities in all treatment and control vessels was determined and the inhibition in cell growth, relative to the control group was determined. Cell densities were used to calculate endpoints in terms growth rate and biomass (ErC50, EbC50 and NOEC values), based on the nominal and measured glyphosate concentrations (average recovery rate was 70.1%) derived from the chemical analysis.

At the start of the test, measured concentrations of glyphosate acid ranged between 68.9 and 80.6% of nominal. At the end of the test, they ranged between 52.0 and 73% of nominal in the (low, mid and high) 4.27, 20.66 and 100 mg test item/L treatments. Glyphosate acid was not detected in the control group.

The validity criteria according to guideline OECD 201 are fulfilled.

The author concluded that 72 h and 96 h ErC50 values for *Pseudokirchneriella subcapitata* exposed to glyphosate isopropylamine salt were 31.70 and 32.01 mg/L, equivalent to 23.48 and 23.71 mg glyphosate acid/L (mean measured). The 72 h and 96 h EbC50 for *P. subcapitata* exposed to glyphosate isopropylamine salt was calculated to be 9.25 and 10.30 mg/L, equivalent to 6.85 and 7.63 mg glyphosate acid/L (mean measured).

RMS considered that the reliability of the 72h ErC50 value is questionable and RMS proposes to consider the 96h ErC50 instead for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate isopropylamine salt
Description:	Light brown liquid
Lot/Batch #:	Tech L 020131
Purity:	62.66 % Glyphosate isopropylamine salt
Vehicle of test material/media and positive control:	Vehicle: SAG medium Positive control:Potassium dichromate

Test organism:

Species:	*Pseudokirchneriella subcapitata (*Chodat, strain: SAG 61.81)
Initial cell concentration	1 x 10^4 cells/mL
Source:	Pflanzenphysiologisches Institut, Göttingen, Germany

Environmental conditions:

Temperature:	21.7 – 25.0°C
Photoperiod:	24 h light
Light intensity	8082 lux
Light quality	Universal white light
pH:	5.7 – 6.2

B. STUDY DESIGN

Experimental dates: 12 July– 19 July 2002 (Biological work)

Experimental treatments

On the basis of the results of a range finding test, the main test was performed with five concentrations, 4.27, 9.39, 20.66, 45.45 and 100 mg test item/L and a negative control (culture medium only). A toxic reference item Potassium dichromate was performed in August 2002.

For each concentration and the control, four vessels were prepared using 300 ml Erlenmeyer flasks each containing 100 mL of control or test medium. The initial cell concentration was 1 x 10^4 cells/mL. The concentrations of glyphosate IPA salt in the test solutions were measured by HPLC as concentrations of glyphosate acid at the start and at the end of the test in the 4.27, 20.66 and 100 mg test item/L treatments. Endpoints were calculated using the average recovery rate of glyphosate achieved over the duration of the test, based on geometric mean measured values achieved for each of the treatment groups. A stability sample was analysed from a test vessel without algae with the highest test item concentration at the end of the exposure period.

To maintain the algae in the suspension, all flasks were shaken continuously over the entire test period (100 ± 5 oscillations/min).

Observations

After 24, 48, 72 and 96 hours of growth, the algal cell densities in the control and test concentration vessels were determined using a Thoma counting chamber with a light microscope and the % growth inhibition (biomass and rate) relative to the control group was determined. This was achieved by plotting the mean value of the cell concentration (converted in log values) against the percentage growth

inhibition to generate dose-response curves for each concentration. The concentrations resulting in 50 % inhibition (ErC50, EbC50), were determined, as well as the NOEC. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured continuously with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
Probit analysis was used to calculate the EC10, EC20, and EC50 values. One-way ANOVA, Cochran´s Test and subsequent Dunnett´s t-test was used to calculate whether there were significant differences between the growth of algae in the controls and the algae exposed to the various test item concentrations to establish NOErC and NOEbC values.

II. RESULTS AND DISCUSSION

A. FINDINGS

In the test, concentrations of glyphosate acid were determined. In stock solutions prepared at test start, measured concentrations were 81.9% of nominal concentrations. In test media at the beginning of the test, mean concentrations were 75.9% of nominal concentrations and at the end of the test (96 h), mean concentrations were 64.2% of nominal with 45.2% found in the stability sample without algae (see table below). The average recovery in all water samples containing algae was 70.1% for Glyphosate isopropylamine salt. Therefore, results are based on mean measured concentrations.

Table B.9.2.6.1-1: Analytical measurements

Test item concentration (nominal) (mg/L)	Glyphosate IPA (nominal) (mg/L)	Glyphosate acid (mean measured) (mg/L)		Glyphosate IPA salt/L (mean measured) (mg/L)		% of nominal	
		-0.5 h	96 h	-0.5 h	96 h	-0.5 h	96 h
500 (Stock solution)	313.30	190.245	-	256.7	-	81.9	-
Control	0	nd	nd	nd	nd	-	-
4.27	2.68	1.554	1.341	2.1	1.8	78.4	67.6
20.66	12.95	6.606	7.007	8.9	9.5	68.9	73.0
100	62.66	37.406	24.164	50.5	32.6	80.6	52.0
100 (stability sample without algae)	62.66	-	20.991	-	28.3	-	45.2

nd = not determined

The ErCx, EbCx and NOEC values are given below based on nominal and arithmetic mean measured concentrations.

Table B.9.2.6.1-2: Percentage inhibition of growth rate and biomass of to *Pseudokirchneriella subcapitata* exposed for 72 and 96 hours to glyphosate isopropylamine salt

Glyphosate isopropylamine salt formulation (nominal) (mg/L)	**Control**	**4.27**	**9.39**	**20.66**	**45.45**	**100.0**
Glyphosate isopropylamine salt (mean measured) (mg/L)[1]		**2.99**	**6.58**	**14.48**	**31.86**	**70.1**
Glyphosate acid (mean measured) (mg/L)[2]		**2.21**	**4.87**	**10.73**	**23.6**	**51.9**
Inhibition growth rate (0-72 h) (%)	-	1.6	6.6*	25.2*	64.8*	61.4*
Inhibition growth rate (0-96 h) (%)	-	-0.9	4.2	17.5*	53.4*	77.1*
Inhibition biomass (0-72 h) (%)	-	11.4	33.5*	70.7*	92.4*	91.9*
Inhibition biomass (0-96 h) (%)	-	3.2	27.1*	68.0*	94.9*	95.9*

* Significantly different from the control at $\alpha = 0.05$

[1] Taken into account the average recovery of 70.1 % for Glyphosate isopropylamine salt.

[2] Taken into account 1.35 ratio stated in the report.

B. OBSERVATIONS

The results of the definitive test show that for algal growth rates, after 72 hours, these were significantly inhibited at nominal concentrations of 9.39 mg test item/L and higher. After 96 hours, significant inhibition was observed at 20.66 mg test item/L and higher.

For biomass, after 72 and 96 hours, there were significant effects observed at nominal concentrations of 9.39 mg test item/L and higher.

In contrast no inhibition of the algae growth was found at or below a nominal concentration of 4.27 mg test item/L.

Table B.9.2.6.1-3: Toxicity of Glyphosate (expressed as IPA salt and Glyphosate acid) to *Pseudokirchneriella subcapitata*

Endpoint		Glyphosate IPA salt (nominal) [95% C.I.] (mg/L)	Glyphosate IPA salt (mean measured) (mg/L)	Glyphosate acid (mean measured) [1] (mg/L)
Growth rate	72 hours			
	0 - 72 h ErC10	8.16 [n.d.; 20.5]		4.23[2]
	0 - 72 h ErC20	14.7 [n.d.; 31.1]		7.6[2]
	0 - 72 h ErC50	45.2 [13.97 ; 1472.2]	31.7	23.5
	0 - 72 h NOEC	4.27	2.99	2.21
	96 hours			
	0 - 96 h ErC10	13.7 [5.5 ; 20.6]		7.11[2]
	0 - 96 h ErC20	20.8 [10.99 ; 28.4]		10.8[2]
	0 - 96 h ErC50	45.7 [34.7 ; 61.3]	32.0	23.7
	0 - 96 h NOEC	9.39	6.58	4.87
Yield	72 hours			
	0 - 72 h EyC10	4.18 [2.13 ; 5.96]		2.17[2]
	0 - 72 h EyC20	6.21 [3.82 ; 8.18]		3.22[2]
	0 - 72 h EyC50	13.2 [10.46 ; 16.6]	9.25	6.85
	0 - 72 h NOEC	4.27	2.99	2.21
	96 hours			
	0 - 96 h EyC10	5.88 [4.5 ; 7.05]		3.05[2]
	0 - 96 h EyC20	8.06 [6.66 ; 9.26]		4.19[2]
	0 - 96 h EyC50	14.7 [13.2 ; 16.3]	10.3	7.63
	0 - 96 h NOEC	4.27	2.99	2.21

[1] The ratio between mean measured concentration in mg glyphosate IPA salt/L and mg glyphosate acid/L is stated as 1.35 in the report.
[2] added by RMS

For the toxic reference item, the 96 h EbC50 was 0.497 mg test item/L and the 96 h ErC50 was 1.721 mg test item/L. These results were in agreement with what was expected on the basis of data shown in EEC Directive 92/69 method C.3.

The biomass in the control cultures increased by a factor of ≥16 (actual value 152.9), the coefficient of variance for section-by-section specific growth rates was ≤35 % (actual values ranged between 0 and 28.0), and the coefficient of variation of average specific growth rates during the whole test period in replicate control was ≤7 % (actual value: 0.8 %). The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSION

Assessment and conclusion by applicant:

The biomass in the control cultures increased by a factor of ≥16 (actual value 152.9), the coefficient of variance for section-by-section specific growth rates was ≤35% (actual values ranged between 0 and 28.0), and the coefficient of variation of average specific growth rates during the whole test period in replicate control was ≤7% (actual value: 0.8%). The validity criteria according to guideline OECD 201 are therefore fulfilled.

The 72 h and 96 h ErC50 values for *Pseudokirchneriella subcapitata* exposed to glyphosate isopropylamine salt were calculated to be 31.70 and 32.01 mg test item/L, corresponding to 23.5 and 23.7 mg a.e./L (arithmetic mean measured). The 72 h and 96 h EbC50 for *P. subcapitata* exposed to glyphosate isopropylamine salt was calculated to be 9.25 and 10.30 mg test item/L, equivalent to 6.85 and 7.63 mg a.e./L (arithmetic mean measured). The 72h NOErC and NOEbC value was 2.21 mg a.e./L, respectively.

The study is considered valid and 72 h NOEC, ErC50, EbC50 values of 2.21, 23.5 and 6.85 mg a.e./L (arithmetic mean measured), respectively, are reliable for risk assessment purposes.

Assessment and conclusion by RMS:

The study is valid.
All validity criteria were met according to guideline OECD 201 (2011) for both 72h and 96h. At 72h, the biomass in the control cultures increased by a factor of ≥16 (actual value 152.9), the coefficient of variance for section-by-section specific growth rates was ≤35% (17.7%), and the coefficient of variation of average specific growth rates during the whole test period in replicate control was ≤7% (actual value: 1.6%). At 96h, the biomass in the control cultures increased by a factor of ≥16 (actual value 328.4), the coefficient of variance for section-by-section specific growth rates was ≤35% (28%) and the coefficient of variation of average specific growth rates during the whole test period in replicate control was ≤7% (actual value: 2.7%).
Geometric mean measured concentrations should have been used for calculations of endpoints. Nevetheless, this would not change the outcome of the study as the nominal concentrations would have been corrected by 69.4% instead of 70.1%. Therefore, arithmetic mean measured concentrations used in the study are accepted by RMS.

The effects measured on biomass as presented in the study summary are based on differences of biomass increase and not on AUC measurements. This in fact corresponds to effects on yield and not on biomass as described in OECD 201 guideline. Therefore, the EbCx calculated in the study have been corrected to EyCx by RMS.
The 95% confidence interval of the 72h ErC50 value of 23.5 mg glyphosate acid/L (mm) is very large. The upper limit of the 95% confidence interval of the 72h ErC50 is far above the range of tested concentrations. Therefore, the reliability of this endpoint is questionable and RMS proposes to consider the 96h ErC50 instead for risk assessment.

72h NOErC = 2.21 mg glyphosate acid/L (mm)

72h ErC10 = 4.23 mg glyphosate acid/L (mm)
72h ErC20 = 7.6 mg glyphosate acid/L (mm)

96h NOErC = 4.87 mg glyphosate acid/L (mm)
96h ErC10 = 7.11 mg glyphosate acid/L (mm)
96h ErC20 = 10.8 mg glyphosate acid/L (mm)
96h ErC50 = 23.7 mg glyphosate acid/L (mm)

72h NOEyC = 2.21 mg glyphosate acid/L (mm)
72h EyC10 = 2.17 mg glyphosate acid/L (mm)
72h EyC20 = 3.22 mg glyphosate acid/L (mm)
72h EyC50 = 6.85 mg glyphosate acid/L (mm)

96h NOEyC = 2.21 mg glyphosate acid/L (mm)
96h EyC10 = 3.05 mg glyphosate acid/L (mm)
96h EyC20 = 4.19 mg glyphosate acid/L (mm)
96h EyC50 = 7.63 mg glyphosate acid/L (mm)

Data point	CA 8.2.6.1/002
Report author	[redacted]
Report year	2003
Report title	MON 78623: a 72-hour toxicity test with the freshwater alga (*Selenastrum capricornutum*)
Report No	139A-311
Document No	-
Guidelines followed in study	OECD Guideline 201 (1984) EU Directive 92/69/EEC, Method C.3. (1992) ASTM Standard Guide 1218-90E (1990)
Deviations from current test guideline by the applicant: See RMS analysis in RMS comment box	*Deviations from the guideline OECD 201 (2011):* *Major:* *- The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 39.8 % instead of <35%*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary

The effects of MON 78623 (K-salt) on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. *P. subcapitata* were exposed to five nominal concentrations encompassing 7.5, 15, 30, 60 and 120 mg test item/L, and the measured concentrations were 7.1, 15, 30, 61 and 122 mg test item/L.

For each concentration, three parallel cultures in 250 ml Erlenmeyer flasks were prepared. The initial cell concentration was 10^4 cells/mL. For the control group, six parallel test vessels were prepared. An additional abiotic replicate at the highest test concentration was included in the experimental design for concentration verification at 72 hours.

After 24, 48 and 72 hours of growth, the numbers of viable cells for each test concentrations and control were determined and the growth inhibition was calculated. At this, concentrations resulting in 50 % inhibition (EC50, ErC50, EbC50), were determined, as well as the NOEC.

EC50, EbC50, ErC50 and the corresponding 95% confidence limits for each 24-hours exposure interval were calculated by non-linear regression.

The results of main test showed that the algae growth was inhibited at the measured concentrations of 61 and 122 mg test item/L. In contrast, no inhibition of the algae growth was found at or below a measured concentration of 30 mg test item/L.

The 72 hours-EC50, EbC50 and ErC50 for *P. subcapitata* exposed to MON 78623 was determined at 69, 74 and 114 mg test item/L. The NOEC was 30 mg test item/L. The validity criteria according to the current guideline OECD 201 were not met, therefore, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	MON 78623, 47.7% Glyphosate
Lot No.:	GLP-0108-11688-F
Chemical purity:	47.7%
Physical state:	Yellow liquid
Expiration date:	October, 2003

Analytical standard:

Identification:	Glyphosate (A.S.)
Lot No.:	GLP-9607-7215-A
Chemical purity:	99.8%
Physical state:	Powder
Expiration date:	January 31, 2003

Vehicle of test material:

Dilution water

Test organism:

Species:	*Pseudokirchneriella subcapitata*, formerly known as *Selenastrum capricornutum*
Initial cell concentration:	10^4 cells/mL
Source:	in-house culture, started from University of Toronto Culture Collection

Environmental conditions:

Temperature:	22.0 – 22.3 °C
Photoperiod:	24 h light
Light intensity:	6500 – 8550 lux

Light quality:	cool-white fluorescent lighting
pH:	8.0 – 8.1 (negative control); 6.9 – 7.8 (highest test concentration)
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 18 October – 21 October 2002

Experimental treatments
Three replicate cultures per test concentration of *P. subcapitata* (initial cell density in each chamber was 1×10^4 cells/mL) were exposed for 72 hours to nominal concentrations of 7.5, 15, 30, 60, and 120 mg test item/L. A negative control group with six replicate cultures was held under the same environmental conditions concurrently. An additional abiotic replicate at the highest test concentration was included in the experimental design for concentration verification at 72 hours. The methods of test solution preparation were stock solution preparation and proportional diluting. The test flasks were shaken continuously at 100 rpm during the test.

Observations
The temperature of a container of water adjacent to the test chambers in the environmental chamber was recorded twice daily during the test using a liquid-in-glass thermometer. Light intensity was measured at five locations surrounding the test flasks on each shaker table at test initiation. The pH of the medium in each treatment and control group was measured at test initiation and at test termination.

Test medium samples were collected from each biological replicate of the treatment and control group for the determination of algal cell densities. Samples were collected at approximately 24-hours intervals during the 72-hours exposure and were held for a maximum of two days under dark, refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Prior to conducting cell counts, the linearity of the instrument response was determined at settings previously established for *P. subcapitata*.

Samples of the test solutions were collected at approximately 0 and 72 hours to measure concentrations of the test substance. At test initiation samples were collected for each treatment and control group prior to addition of the algae. At test termination, the biological replicates from each respective treatment and control group were pooled and then sampled. The 120 mg test item/L equivalent to 57.24 mg glyphosate/L abiotic replicate was sampled at test termination to determine the stability' of the test substance under the conditions of administration. All samples were collected in glass vials and processed immediately for analysis.

Statistical calculations:
Cell densities, areas under the growth curve, growth rates and percent inhibition values were calculated using SAS System for Windows (Version 8.02). Cell densities, areas under the growth curve and growth rates were analysed statistically to estimate EC50 values and the corresponding 95% confidence limits for each 24-hours exposure interval. All EC50 values were calculated by non-linear regression.
The cell density, area under the growth curve and growth rate data were evaluated for normality and homogeneity of variance (p=0.05) using the Shapiro-Wilk's and Levene's tests, respectively. Since the data were normal with homogeneous variances, the treatment groups were compared to the negative control using ANOVA and Dunnett's test (p=0.05). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOEC relative to each parameter at 72 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50, EbC50, ErC50 and NOEC values are given below based on mean measured concentrations.

Table B.9.2.6.1-4: Toxicity of MON 78623 to *Pseudokirchneriella subcapitata*

Endpoint	MON 78623 [mg test item/L]
EC50 (cell density) and 95% Confidence Limits	69 (62 – 77)
EbC50 (biomass) and 95% Confidence Limits	74 (67 – 83)
ErC50 (growth rate) and 95% Confidence Limits	114 (111 – 118)
NOEC (cell density)	30
NOEC (biomass)	30
NOEC (growth rate)	30

Concentrations of MON 78623 in the samples were determined using a HPLC (UV detector at 500nm). Calibration standards of Glyphosate, ranging in concentration from 2.00 to 20.0 mg glyphosate/L, were prepared in freshwater algal medium using a stock solution of Glyphosate in NANOpure® water. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The method limit of quantitation (LOQ) for these analyses was defined as 4.19 mg test item/L equivalent to 2.00 mg glyphosate/L. The analytical results are given below.

Table B.9.2.6.1-5: Analytical measurements

MON 78623 nominal [mg /L]	Sampling time [hours]	MON 78623 measured [mg test item/L]	Percent of nominal [%]	MON 78623 mean measured [mg test item/L]	Mean percent of nominal [%]
-	0	< LOQ	-	-	-
	72	< LOQ	-		
7.5	0	6.20	82.7	7.1	94.7
	72	8.03	107		
15	0	14.7	98.3	15	100
	72	15.7	104		
30	0	29.5	98.3	30	100
	72	31.2	104		
60	0	59.3	98.8	61	102
	72	62.1	104		
120	0	119	99.3	122	102
	72	124	103		
120 (Abiotic)	72	125	104	-	-

Although the determined concentrations of test item in test medium always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using the mean determined concentrations of the test item.

B. OBSERVATIONS

The results of main test showed that the algae growth was inhibited at the measured concentrations of 61 and 122 mg test item/L corresponding to 28.95 and 57.96 mg glyphosate/L. In contrast, no inhibition of the algae growth was found at or below a measured concentration of 30 mg test item/L corresponding to to 14.48 mg glyphosate/L.

Table B.9.2.6.1-6: Percentage inhibition of growth rate and biomass to P. subcapitata exposed for 72 hours to MON 78623

	Control	MON 78623 [mg test item/L]				
	-	7.1	15	30	61	122
Mean number of algae cells (10000/ml)	81.3645	92.6914	97.6039	86.9339	54.8190*	7.4236*
Inhibition growth rate (0-72 h) [%]	-	-3.1	-4.3	-1.7	9.0*	54*
Inhibition biomass (0-72 h) [%]	-	-12	-17	-6.1	26*	88*

* There were statistically significant differences (p<0.05) in comparison to the negative control replicates.

III. CONCLUSIONS

The 72 h ErC50 for *Pseudokirchneriella subcapitata* exposed to MON 78623 was determined at 114 mg test item/L. The 72 h EbC50 for *P. subcapitata* exposed to MON 78623 was 74 mg test item/L. The 72 h EC50 for *P. subcapitata* exposed to MON 78623 was 69 mg test item/L. Significant effects of MON 78623 on the growth of *P. subcapitata* were found from a concentration > 30 mg test item/L. The NOEC was 30 mg test item/L.

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	81.4
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	39.8%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	3.4%

The biomass in the control cultures increased by a factor of ≥16 (actual: 81.4), the coefficient of variance for section specific growth rates exceeded 35% (actual: 39.8%), for the whole test period it was ≤ 7% (actual 3.4%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The study is not valid as the mean coefficient of variation for section-by-section specific growth rates in the control exceeded the trigger of 35% according to current OECD 201 guideline (2011).

Data point	CA 8.2.6.1/003
Report author	[redacted]
Report year	2000
Report title	Acute toxicity of glifosate tecnico [redacted] to *Selenastrum capricornutum*
Report No	RF-D2.44/99
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1993)
Deviations from current test guideline by the applicant: See RMS analysis in RMS comment box	*Deviations from the guideline OECD 201 (2011):* *Major:* *- Analytical verification of test item only performed at the start of the test.in samples of test medium and stock solution (both >80% of nominal).*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Summary

The toxicity of glyphosate technical to the green alga *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) was determined in a 96-hour, static test. The test comprised 7 nominal concentrations of glyphosate (nominal 5.6, 10, 32, 56, 100, 320, and 560 mg test item/L, corresponding to initial measured concentrations of 5.74, 9.81, 33.48, 58.55, 104.17, 325.42, and 585.52) and a control (untreated culture medium) without test item. The test vessels were 250 mL glass Erlenmeyer flasks containing 100 mL of test solution.

Three replicate vessels were prepared for each test concentration and for the control group. Each replicate test vessel was inoculated with an initial cell density of 1.6×10^4 cells/mL. After 1, 2, 3, and 4 days, samples were removed from each test and control vessel and the algal cell densities were determined by cell counting. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily. The concentrations of glyphosate technical in the test solutions were measured at the start of the test. The measured test concentration values were used for the calculation and reporting of all results.

The effective concentration of glyphosate technical causing 50% inhibition of growth in *Pseudokirchneriella subcapitata* after 96 hours when compared to the control was 114.05 mg test item/L, the no observed effect concentration (NOEC) was 104 mg test item/L (initial measured concentrations).

The validity criteria according to the current guideline OECD 201 were met. However, analytical work was not performed throughout the test, as required per current test guidelines. Therefore, this study is considered supportive only.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	037-919-113
Purity:	954.9 g/kg
Vehicle of test material/media:	Cell growth medium

Test organism:

Species:	Green algae *Pseudokirchneriella subcapitata,* UTEX 1648
Initial cell concentration	1.6×10^4 cells/mL
Source:	UTEX – The culture collection of algae at the University of Texas at Austin, Texas, USA
Acclimatisation period:	4 days

Environmental conditions:

Temperature:	24.3-24.4°C
Photoperiod:	Continuous illumination
Light intensity:	7933 lux
pH:	7.17 – 7.22 at 0 hour 7.46 – 9.31 at 72 hour

B. STUDY DESIGN

Experimental dates: 25 October -12 November 1999

Experimental treatments

The toxicity of glyphosate to the green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour, static test. The test comprised 7 nominal concentrations of glyphosate (nominal 5.6, 10, 32, 56, 100, 320, and 560 mg test item/L, corresponding to initial measured concentrations of 5.74, 9.81, 33.48, 58.55, 104.17, 325.42, and 585.52 mg test item/L) and a control consisting of culture medium without test item. The test vessels were 250 mL glass Erlenmeyer flasks containing 100 mL of test solution.

A primary stock solution of nominal concentration of 10000 mg test item/L was prepared by dissolving 1.0 g glyphosate in 100 mL distilled and deionised water. From this initial solution, following stock solutions were prepared: 10, 100, and 1000 mg test item/L. Appropriate aliquots of these stock solutions were diluted to prepare the test concentrations. 100 mL of the appropriate test solution were dispensed to each test and blank vessel. The test comprised 3 replicates of the control (untreated culture medium) and 3 replicates of each concentration of the test item.

Each replicate test vessel was inoculated with a cell density of 1.6×10^4 cells/mL. The culture vessels were incubated at 24.3 - 24.4°C under continuous illumination for 96 hours. During incubation, the algal cells were kept in suspension by continuous shaking.

Observations

After 1, 2, 3 and 4days, samples were removed from each test and control vessel and the algal cell densities were determined by cell counting using a Neubauer improved haemacytometer and a phase-contrast microscope. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a minimum-maximum thermometer. The concentrations of glyphosate in the test solutions were measured at the start of the test

only. The effective concentration was within acceptable limits of nominal concentration (80%) for all tested concentrations.

Statistical calculations
The computer program used was STATGRAPHICS – Statistical Graphic System.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} (96 h), NOEC and LOEC values are given below based on initial measured concentrations.

Table B.9.2.6.1-7: Toxicity of glyphosate *to Pseudokirchneriella subcapitata*

Endpoint	Glyphosate [mg test item/L]
96-h EC_{50} (95% CI)	114.05 (94.04 - 131.49)
96–h NOEC	104.17
96–h LOEC	325.42

CI = confidence interval

B. OBSERVATIONS

The effective concentration of glyphosate technical causing 50% inhibition of growth after 96 hours when compared to the control was 114.05 mg test item/L, the no observed effect concentration (NOEC) was 104.17 mg test item/L. No morphological changes were observed after 96 hours of exposure to glyphosate technical.

Table B.9.2.6.1-8 Mean cell densities and Percentage of inhibition of cell yield of *Pseudokirchneriella subcapitata* exposed for 72 and 96 hours to glyphosate

	Control	Glyphosate technical [mg/L]						
Test parameters	-	**5.6**	**10**	**32**	**56**	**100**	**320**	**560**
Mean cell densities (0-96 h) (× 10000 cells/mL)	740	732	723	723	707	473	48.4	23.4
Mean yield inhibition (0-96 h) [%]		99	98	98	96	64	7	3
Mean cell densities (0-72 h) (× 10000 cells/mL)	307	290	248	215	223	173	23.4	23.4
Mean yield inhibition (0-72 h) [%]		94	81	70	73	57	8	8

The biomass in the control cultures increased by a factor of ≥16, the coefficient of variance for section specific growth rates was ≤35%, for the whole test period it was ≤7%. The validity criteria according to guideline OECD 201 are therefore fulfilled.

III. CONCLUSIONS

The 96 h EyC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate technical was calculated to be 114.05 mg test item/L, the no observed effect concentration (NOEC) was 104 mg test item/L (initial measured concentrations).

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	192
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	10%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	0.9%

The biomass in the control cultures increased by a factor of ≥16 (actual: 192), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 10%) and the coefficient of variance for the whole test period it was ≤ 7% (actual: 0.9%). The validity criteria according to the current guideline OECD 201 were met. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required per current test guidelines. As there are other studies with more sensitive endpoints available, this study is considered supportive only.

Nevertheless, endpoints were recalculated.

A statistical re-evaluation addressing EC10, EC20, EC50, NOEC and LOEC was performed (Positon Paper No. 110054-001). Endpoints are based on nominal concentrations.

Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate [mg a.e./L]	
	Yield	Growth rate
EC10 (95% CI)	5.54 (2.99 – 8.68)	62.6 (40.4 – 84.6)
EC20 (95% CI)	14.6 (9.40 – 20.5)	132 (100 – 161)
EC50 (95% CI)	75.9 (56.4 – 105)	469 (401 – 568)
NOEC	5.6	5.6
LOEC	10	10

Assessment and conclusion by RMS:

All validity criteria were met according to OECD 201 (2011) guideline.
However, the analytical measurements were only made at test start in the tested concentrations and stock solutions, and not during or at the end of the test as recommended in the guideline. In Table B.9.2.6-8, only the effects on yield are presented. The effects on growth rates were checked by RMS and are presented below:

	Control	Glyphosate technical [mg/L]						
Test parameters	**-**	**5.6**	**10**	**32**	**56**	**100**	**320**	**560**

Mean growth rate (0-96 h)	1.53	1.53	1.53	1.53	1.52	1.42	0.85	0.67
Growth rate compared to control (0-96 h) [%]		99.8	99.6	99.6	99.2	92.7	55.6	43.7
Mean growth rate (0-72 h)	1.75	1.73	1.68	1.63	1.65	1.56	0.89	0.89
Growth rate compared to control (0-72 h) [%]		98.9	96.0	93.2	94.0	89.1	51.0	51.0

A statistical analysis of the endpoints of this study based on yield and growth rate was conducted by applicant (see study summary and RMS opinion below).

As it is not known if the concentrations were maintained or not, RMS considers this study as supportive.

Data point	CA 8.2.6.1/004
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study RF-D2.44/99 on the toxicity of glifosate tecnico [redacted] to *Selenastrum capricornutum* under static conditions
Report No	110054-001
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1993)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

Summary

A statistical evaluation addressing the calculation of valid EC10, EC20 and EC50 as well as NOEC values was conducted for the algae study RF-D2.44/99 ([redacted] 2000) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).
Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. The calculated EC_{10}, EC_{20} and EC_{50} values are 62.6, 132, and 469 mg/L for growth rate and 5.54, 14.6, and 75.9 mg a.e./L for yield, respectively. The NOEC for growth and yield was determined to be 5.6 mg a.e./L. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required as per current test guidelines. As there are other studies with more sensitive endpoints available, this study is considered supportive only and is not used for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number:	RF-D2.44/99
Author:	[redacted]
Substance:	Glyphosate
Title:	Acute toxicity of glifosate tecnico [redacted] to *Selenastrum capricornutum*
Completion date:	03-01-2000
Test guideline(s):	OECD 201 (1993)
GLP: Yes	
Testing facility:	BIOAGRI Laboratorios, Piracicaba, SP. Brasil
Sponsor:	NUFARM DO BRASIL Ltda., Curitiba, PR., Brasil

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC10, EC20, and EC50 as well as the NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study RF-D2.44/99 ([redacted] 2000) was statistically evaluated for the effects of glyphosate on the organism *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*, also formerly known as *Pseudokirchneriella subcapitata*). The organisms were exposed for 96 hours to the following concentrations of glyphosate 5.6, 10, 32, 56, 100, 320, and 560 mg test item/L (nominal) and corresponding to initial measured concentrations 5.74, 9.81, 33.48, 58.55, 104.17, 325.42, and 585.52 mg test item/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive Effect Concentrations that have 10, 20 and 50% effects on yield and growth rate of the test subjects (EC10, EC20 and EC50), a logit analysis using linear weighted regression was performed. For growth rate, a logit analysis with linear maximum likelihood regression was used.

NOEC was determined by Welsh-t-test After Bonferroni-Holm (one-sided smaller, $p = 0.05$).
Analyses were performed.

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Results are provided in the table below:

Table B.9.2.6.1-9: Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	192
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	10%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	0.9%

The validity criteria according to the current guideline OECD 201 were met. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required per current test guidelines.

For yield, the parameters for the logit model are estimated as slope b: 1.93240; Intercept a: -3.63371.

For growth rate, the parameters for the probit model are estimated as slope b: 2.51249; Intercept a: -6.71063.

According to the statistical parameters; Chi2(13) = 0.283521; p(Chi²): 1.000; F(1,19) = 120.416; p(F) <0.001; r²: 0.864 for yield; and Chi2(13) = 0.04958; p(Chi²): 1.000; F(1,19) = 107.785; p(F) <0.001; r²: 0.850 for growth rate. Based on these values the EC10, EC20 and EC50 for yield and growth rate calculations should be considered valid.

The obtained EC_{10}, EC_{20} and EC_{50}, and NOEC values for the effect of Glyphosate on growth rate and yield of *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata* or formally known as *Pseudokirchneriella subcapitata*) are presented in the table below.

Table B.9.2.6.1-20: Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate technical [mg a.e./L]	
	Yield	Growth rate
EC_{10} (95% CI)	5.54 (2.99 – 8.68)	62.6 (40.4 – 84.6)
EC_{20} (95% CI)	14.6 (9.40 – 20.5)	132 (100 – 161)
EC_{50} (95% CI)	75.9 (56.4 – 105)	469 (401 – 568)
NOEC	5.6	5.6
LOEC	10	10

CI = confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:

The validity criteria according to the current guideline OECD 201 were met and this study is considered valid in view of parameters for increase of biomass, mean coefficient of variation for section-by section specific growth rate, and coefficient of variation of average specific growth rates. However, analytical work was performed only at test initiation, yet not throughout the test nor at test end, as required as per current test guidelines. As there are other studies with more sensitive endpoints available, this study is considered supportive only and is not used for risk assessment.

Nevertheless, the calculated EC_{10}, EC_{20} and EC_{50} values are 62.6, 132, and 469 mg a.e./L (nominal) for growth rate and 5.54, 14.6, and 75.9 mg a.e./L (nominal) for yield, respectively. The statistical parameters showed that these values can be considered reliable. The nominal based NOEC was determined to be 5.6 mg a.e./L for yield and growth rate.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. Nevertheless, as stated above, RMS considers this study as supportive.

Data point	CA 8.2.6.1/005
Report author	[redacted]
Report year	1995
Report title	Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*
Report No	AB0503/B
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline by the applicant: See RMS analysis in RMS comment box	*Deviations from the guideline OECD 201 (2011):* *Minor:* *- Initial nominal cell density of 3 × 10^3 cells/mL, was below the recommended density of 5 × 10^3 – 10^4 cells/mL for P. subcapitata, however validity criteria were met*.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The toxicity of glyphosate acid to the green alga *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) was determined in a 120-hour, static test conducted at six nominal glyphosate acid concentrations (5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control prepared using culture medium without test item.

Six replicate vessels were prepared for the control group with three replicate vessels prepared for each concentration of glyphosate acid. Each replicate test vessel was inoculated with 0.370 mL of the

inoculum culture to give a nominal cell density of 3×10^3 cells/mL. The culture vessels were incubated at 24 ± 1°C in an orbital incubator (vessels shaken at 100 rpm) under continuous illumination for 120 hours.

The algal cell densities were determined after 1, 2, 3, 4, and 5 days. Test and control group media pH values were determined at the beginning and end of test, with temperature measured hourly. Glyphosate acid concentrations in test solutions were measured at the start and at the end of the test. The mean measured glyphosate acid concentrations ranged from 100 to 111% of the nominal values.
Endpoints were recalculated by applicant in a new statistical analysis (see study summary and RMS opinion below).
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%
Vehicle of test material/media:	Cell growth medium

Test organism:

Species:	Green algae *Pseudokirchneriella subcapitata* Korshikov
Initial cell concentration	3×10^3 cells/mL
Source:	Brixham Environmental Laboratory culture from strain ATCC 22662

Environmental conditions:

Temperature:	24.1 - 24.2°C (measured by thermometer). The hourly temperature measured automatically remained within 24 ± 1°C
Photoperiod:	Continuous illumination
Light intensity:	5030 lux
pH:	3.5 – 7.5 at the start of the test
	3.6 – 8.9 at the end of the test

B. STUDY DESIGN

Experimental dates: 7 August - 12 August 1995

Experimental treatments
The toxicity of glyphosate acid to the green alga *Selenastrum capricornutum* (currently known as *Raphodocelis subcapitata*) was determined in a 120-hour, static test, conducted at six nominal glyphosate acid concentrations of 5.6, 10, 18, 32, 56, and 100 mg test item/L, and a control consisting of culture medium without test item. The test vessels were 250 mL conical glass flasks containing 100 mL of test or control solution. The stock solution (nominal concentration of 100 mg a.s./L) was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 5.6, 10, 18, 32, and 56 mg test item/L. To each test and blank vessel 100 mL of the appropriate test solution were dispensed. The test was performed in six replicate cultures of the culture medium control and three replicate cultures of each

concentration of glyphosate acid.
Each replicate test vessel was inoculated with 0.370 mL of the inoculum culture to give a nominal cell density of 0.3×10^4 cells/mL. The culture vessels were incubated at 24 ± 1°C under continuous illumination for 120 h. During incubation, the algal cells were kept in suspension by continuous shaking using an orbital incubator (oscillating at 100 rpm).

Observations
The algal cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was monitored continuously with readings recording hourly with an automatic recording system. The concentrations of glyphosate acid in the test and control solutions were measured at the start and at the end of the test.

Statistical calculations
One-way analysis of variance, and Dunnett's post-hoc test to determine the NOEC. ECx values were evaluated by linear regression against log concentration.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.2.6.1-31: Toxicity of glyphosate acid to *Selenastrum capricornutum*

Endpoint	Glyphosate acid [mg test item /L]	
	Growth rate	**Biomass**
72-h EC_{50} (95% CI)	19 (14 - 25)	18 (13 - 23)
72–h NOEC	10	10
72–h LOEC	18	18
120-h EC_{50} (95% CI)	21 (16 - 28)	17 (13 - 22)
120-h NOEC	10	10
120-h LOEC	18	17

CI= Confindence interval

The mean measured concentrations of glyphosate acid ranged from 100 to 111% of the nominal values. On the basis of the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

B. OBSERVATIONS

Table B.9.2.6.1-42 Mean cell densities and percentage of inhibition of cell growth of *Selenastrum capricornutum* exposed for 72, 96 and 120 hours to glyphosate

	Control	Glyphosate acid [mg test item/L]					
Test parameters	-	**5.6**	**10**	**18**	**32**	**56**	**100**
Mean cell densities (0-72 h) (× 10000 cells/mL)	73.4	79.1	74.5	2.05	0.143	0.021	0.033
Mean cell densities (0-96 h) (× 10000 cells/mL)	312	314	311	2.60	0.178	0.070	0.045
Mean cell densities (0-120 h) (× 10000 cells/mL)	567	605	568	4.20	0.478	0.138	0.172
Mean area under growth curve (0-72 h) [%]	-	108	104	8	-1	-1	-1
Mean area under growth curve (0-96 h) [%]	-	103	101	2	0	0	0
Mean area under growth curve (0-120 h) [%]	-	104	100	1	0	0	0
Mean growth rate (0-72 h) [%]	-	101	100	35	-13	-48	-40
Mean growth rate (0-96 h) [%]	-	100	100	31	-7	-21	-27
Mean growth rate (0-120 h) [%]	-	101	100	35	6	-10	-7

III. CONCLUSION

The 72-hour E_bC_{50} and E_rC_{50} for *Selenastrum capricornutum* exposed to glyphosate acid were determined to be 18 and 19 mg test item/L, respectively. The 72-hour NOE_bC and NOE_rC values were 10 mg test item/L, respectively. The 120-hour E_bC_{50} and E_rC_{50} were calculated to be 17 and 21 mg test item/L. The 120-hour NOE_bC and NOE_rC were 10 mg test item/L each.

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	245
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	9.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.6%

The biomass in the control cultures increased by a factor of ≥16 (actual: 245), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 9.1%) and the coefficient of variance for the whole test period it was ≤ 7% (actual: 1.6%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10}, EC_{20}, and EC_{50} was performed (Positon Paper No. CA 8.2.6.1/006).

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.s./L]	
	Yield	Growth rate
EC_{10} (95% CI)	4.84 (2.07 – 7.80)	5.74 (3.65 – 7.87)
EC_{20} (95% CI)	7.59 (3.93 – 11.3)	8.91 (6.25 – 11.6)
EC_{50} (95% CI)	16.4 (10.9 – 23.0)	18.9 (14.9 – 23.7)

CI = confidence interval

Re-calculated EC_{10}, EC_{20} and EC_{50} values based on nominal test concentrations:
The 72-hour NOE_bC and NOE_rC values were provided by the study report as 10 mg a.s./L, based on glyphosate acid.

Assessment and conclusion by RMS:

The study is valid. All validity criteria were met according to OECD 201 (2011) guideline.
A statistical analysis of the endpoints of this study was conducted by applicant and is accepted by RMS (see study summary and RMS opinion below). ECx values at 96h and 120h are not considered necessary as they are not recommended in the OECD 201 guideline and in addition, they are expected to be in the same range as the 72h ECx values. RMS noted that pH values decreased depending on the concentrations in glyphosate acid. The first concentration to show effects is 18 mg/L, concentration in which pH ranged between 5.9 and 6.4 throughout the test. To be able to distinguish effects due to the acidification of the media from other effects, a test with and without adjustment would have been the most suitable option. Nevertheless, it is RMS opinion that the adjustment of pH may however mask the intrinsic biochemical characteristics of glyphosate acid. It should be noted

that the study was already considered in previous assessment (DAR/RAR). In addition, the OECD 201 guideline recommend a specific pH range only for controls and not for the test item. Overall, a test without pH adjustment is considered relevant by RMS in this case.

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.6.1/006
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL5550/B on the toxicity of Glyphosate acid to *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) under static conditions
Report No	110054-002
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP was not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

Summary

A statistical evaluation addressing the calculation of valid 72-h EC10, EC20 and EC50 values was conducted for the study BL5550/B ([redacted] 1995) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011). Analyses were performed using software ToxRatPro Version 3.3.0.
The validity criteria according to the current guideline OECD 201 (2011) were met.
The author concluded that the calculated EC_{10}, EC_{20} and EC_{50} values are 4.84, 7.59 and 16.4 mg a.e./L (nominal), respectively for yield and 5.74, 8.91 and 18.9 mg/L (nominal), respectively for growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.
RMS recalculated the 72h-ErC50 and found an ErC50 of 17.3 mg/L (95% CI : 15.1 – 17.6 mg/L) which is considered acceptable for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: AB0503/B
Author: [redacted]
Substance: Glyphosate acid
Title: Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum*
Completion date: 15-Aug-1995
Test guideline(s): OECD Guideline No. 201 (1984); US EPA Guideline 540/09-82-020 (1982)

	Re-evaluated according to OECD 201 (2011)
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Testing facility:	Brixham Environmental Laboratory, Brixham Devon, UK
Sponsor:	ZENECA Agrochemicals, Surrey, UK
Sponsor:	ZENECA Agrochemicals, Surrey, UK

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC_{10}, EC_{20}, and EC_{50} values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study BL5550/B ([redacted] 1995) was statistically evaluated for the effects of Glyphosate acid on the organism *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*). The organisms were exposed for 120-hours to the following concentrations of Glyphosate acid: 5.6, 10, 18, 32, 56, and 100 mg test item/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

Statistical calculations
The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

The data was checked for normality using Shapiro-Wilk's Test on Normal Distribution for all time points (p=0.01). Subsequently, for determination of outliers, The Dixon & Hartley outlier test was performed for parametric data (24-h and 48-h replicates), and Hampel Outlier test for non-parametric data (72-h replicates). Only if an outlier was detected repeatedly for a given replicate, it was excluded from subsequent analyses.

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects (EC_{10}, EC_{20} and EC_{50}), a logit analysis was performed and outlier excluded where applicable.

Furthermore, results of the original report were reviewed, which determined the NOEC.

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Results are provided in the table below:

Table B.9.2.6.1-53: Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	245
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	9.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.6%

Outlier test:
Data sets for 24 and 48 hours follow a normal distribution, while the 72 hour dataset is not normally distributed.
According to Dixon & Harley outlier test and Hampel outlier test, the following outliers were determined:

Time point	Test concentration	Replicates
24h	No outliers detected	
48h	32 mg/L 56 mg/L	Replicate 1 Replicate 2
72h	32 mg/L 100 mg/L	Replicate 1 Replicate 2

As replicate 1 in the test concentration of 32 mg/L resulted in being an outlier at 48 as well as 72 hours, this replicate is excluded from further statistical analysis.

The mean measured concentrations of glyphosate acid ranged from 100 to 111% of the nominal values. On the basis of the analytical results the nominal test concentration values were used for the calculation of all results.

For yield at 72 hours, the parameters for the logit model are estimated as slope b: 4.14430; intercept a: -5.03349.

For growth rate at 72 hours, the parameters for the logit model are estimated as slope b: 4.24735; Intercept a: -5.41977.

Statistical parameters for goodness fit of the logit model are: Chi2(15) = 0.473; p(Chi2): 1.000; F(1,15) = 91.681, p(F) <0.001; R2 = 0.859 the EC10 , EC20 and EC50 for growth rate and Chi2(15) = 1.011; p(Chi2): 1.000; F(1,15) = 40.874 p(F) <0.001; R2 = 0.732 the EC10 , EC20 and EC50 for yield, calculations should therefore be considered valid.

The obtained EC_{10}, EC_{20} and EC_{50} values for *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) are presented in the table below.

Geometric mean measured test concentrations ranged from 100 to 111% of nominal. Therefore, all results are based on nominal test concentrations.

Table B.9.2.6.1-64: Re-calculated EC_{10}, EC_{20}, EC_{50} values based on nominal test concentrations:

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.e./L]	
	Yield	Growth rate
EC_{10} (95% CI)	4.84 (2.07 – 7.80)	5.74 (3.65 – 7.87)
EC_{20} (95% CI)	7.59 (3.93 – 11.3)	8.91 (6.25 – 11.6)
EC_{50} (95% CI)	16.4 (10.9 – 23.0)	18.9 (14.9 – 23.7)

CI = confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC_{10}, EC_{20} and EC_{50} values are 4.84, 7.59 and 16.4 mg a.s./L (nominal), respectively for yield and 5.74, 8.91 and 18.9 mg a.s./L (nominal), respectively for growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:
The statistical analysis is considered acceptable. RMS agrees with the outliers identified by applicant and ECx calculations. RMS noted that the results indicated 65% inhibition of mean growth rate at 18 mg/L. Thus the 72h-ErC50 was expected to be between 10 and 18 mg/L. After recalculation with RegTox Tool using bootstrap calculations, RMS found an ErC50 of 17.3 mg/L (95% CI : 15.1 – 17.6 mg/L) which is considered acceptable for risk assessment.

72h NOErC = 10 mg glyphosate acid/L (nom)
72h ErC10 = 5.74 mg glyphosate acid/L (nom)
72h ErC20 = 8.91 mg glyphosate acid/L (nom)
72h ErC50 = 17.3 mg glyphosate acid/L (nom)

72h NOEyC = 10 mg glyphosate acid/L (nom)
72h EyC10 = 4.84 mg glyphosate acid/L (nom)
72h EyC20 = 7.59 mg glyphosate acid/L (nom)
72h EyC50 = 16.4 mg glyphosate acid/L (nom)

Data point	CA 8.2.6.1/007
Report author	[redacted]
Report year	1995
Report title	Fresh Water Algal Growth Inhibition Test with Glyfosaat
Report No	141896
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): *Major (proposed by applicant):* *- The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 56.7% instead of <35% Validity criteria was not met.* RMS recalculated CV section-by-section considering the recommendations of EFSA Supporting publication 2015:EN-924 (Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology). Re-calculated CV is lower than 35%.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid (applicant) / Valid (RMS)

Summary

The effects of glyphosate on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. After a range-finding test *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations encompassing 10, 18, 32, 56 and 100 mg test item/L and a blank control.
For each test concentration and the control group, three (test concentrations) or six (control) replicates with 50 mL test solution and an initial cell density of 1×10^4 cells/mL were prepared in 100 mL vessels. Additionally, for the highest test concentration one replicate without algae was provided.
After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements and a linear calibration curve relating extinction and cell density.

The concentrations resulting in 50% reduction of growth rate (E_rC_{50}) and 50% inhibition of cell growth (E_bC_{50}) were determined, as well as the associated NOEC values.

Results showed glyphosate inhibited cell growth of the fresh water algae *Pseudokirchneriella subcapitata* increasingly with increasing concentrations, resulting in an almost complete inhibition at 56 and 100 mg test item/L. A significant reduction of growth rate was observed at 56 and 100 mg test item/L. Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and the applicant considered this study as not valid for risk assessment purposes.
RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	22021
Purity:	96%
Vehicle of test material/media and positive control:	Vehicle: Dilution water (ISO-medium) Positive control: Potassium dichromate (K2Cr2O7)

Test organism:

Species:	*Pseudokirchneriella subcapitata*, strain: CCAP 278/4
Initial cell concentration	1×10^4 cells/mL
Source:	In-house culture
Acclimatisation period:	Not stated

Environmental conditions:

Temperature:	22.0°C
Photoperiod:	24 h light
Light intensity:	7000 - 8000 lux
pH:	Control (0 – 72 h): 8.1 – 8.2 10 mg/L (0 – 72 h): 7.8 – 7.9 18 mg/L (0 – 72 h): 7.3 – 7.8 32 mg/L (0 – 72 h): 6.5 – 7.6 56 mg/L (0 – 72 h): 5.9 – 6.5 100 mg/L (0 – 72 h): 4.7 – 4.9
Hardness:	24 mg CaCO3/L

B. STUDY DESIGN

Experimental dates: 28 March – 14 April 1995

Experimental treatments

Prior to the main test, a range-finding test was performed with concentrations of 0.01, 0.1, 1, 10 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with five concentration ranges, 10, 18, 32, 56 and 100 mg test item/L. In addition, algae were exposed to test medium without test substance or other additives (blank control). The test solutions were prepared using ISO-medium.

The culture vessels were incubated on a shaking plate over several generations for 72 hours. During the incubation, the algal cells were kept in suspension by continuous shaking. For each concentration, three parallel cultures were prepared in 100 ml all-glass vessels. To each test vessel, 50 mL of the test item preparation were added with an initial cell density adjusted to 1×10^4 cells/mL. Additionally, for the highest test concentration one replicate without algae was provided. For the control group, six parallel test vessels were prepared.

Observations
After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements and a linear calibration curve relating extinction and cell density.
The concentrations resulting in 50% and 10% reduction of growth rate (E_rC_{50} and E_rC_{10}) and 50% and 10 % inhibition of cell growth (E_bC_{50} and E_bC_{10}) were determined, as well as the associated NOEC values.
The pH-values of the test solutions were measured at test initiation and test termination. The temperature was controlled daily in a temperature-control vessel.
Analytical control measurements of the actual concentration of the test item were performed by mean of HPLC analysis, using samples taken from three representative concentrations, 10, 32 and 100 mg test item/L.

Statistical calculations
The calculation of the EC_{50} and EC_{10} values was based on the percentages of growth inhibition and the percentages of growth rate reduction versus the (log) concentration using the linear regression method.

II. RESULTS AND DISCUSSION

A. FINDINGS

E_rC_{50}, E_bC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.6.1.1-7: Toxicity of glyphosate to *Pseudokirchneriella subcapitata*

Endpoint (0 – 72 hours)	Glyphosate [mg test item/L]
E_rC_{50} (95% CI)	54 (51 - 58)
E_bC_{50} (95% CI)	48 (43 - 54)
E_rC_{10} (95% CI)	33 (n.d.- 36)
E_bC_{10} (95% CI)	18 (13 - 22)
NOE_rC	32
NOE_bC	10

CI = confidence interval

Analytical data: Analytical control measurements were performed on three representative concentrations. At test initiation, 106%, 109% and 108% of the test item were recovered for the nominal concentrations of 10, 32 and 100 mg test item/L, respectively. At test termination, 103%, 108% and 111% of the test item were recovered for the nominal concentrations of 10, 32 and 100 mg test item/L, respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.
Reference item: The 72-hour E_bC_{50} was 0.69 mg reference item/L, the 72-hour E_rC_{50} was 1.32 mg reference item/L.

B. OBSERVATIONS

Glyphosate inhibited cell growth of the fresh water algae *Pseudokirchneriella subcapitata* increasingly with increasing concentrations, resulting in an almost complete inhibition at 56 and 100 mg test item/L. A significant reduction of growth rate was observed at 56 and 100 mg test item/L.

Table B.9.2.6.1.1-8: Percentage reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to glyphosate

Test parameters (0 – 72 hours)	Control	Glyphosate [mg test item/L]				
	-	10	18	32	56	100
Mean cell densities (× 10000 cells/mL)	57.4	52.3	49.3	47.8	5.3	1.2
Cell growth rate reduction [%]		2.3	3.7	4.5	58.9	96.0
Cell number inhibition [%]		7.1	9.4	19.9	81.6	96.7

In the control the cell density increased by an average factor of 57 within three days. Analysis of samples taken from the solution without algae showed that the actual exposure concentration remained above 80 % relative to the initial concentration. Further, all test conditions remained within the ranges prescribed by the protocol.

III. CONCLUSION

Under the conditions of the present study the nominal based 72 hours E_rC_{50} and E_bC_{50} for *Pseudokirchneriella subcapitata* exposed to glyphosate were calculated to be 54 mg test item/L and 48 mg test item/L, respectively. The NOE_rC and NOE_bC were determined to be 32 mg test item/L and 10 mg test item/L, respectively.

Assessment and conclusion

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	57.5
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	56.7%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	3.4%

The biomass in the control cultures increased by a factor of ≥16 (actual: 57.5), the coefficient of variance for section specific growth rates exceeded 35% (actual: 56.7%), for the whole test period it was ≤ 7% (actual: 3.4%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:
RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline.
The biomass in the control cultures increased by a factor of 57.5, the mean coefficient of variation for section-by-section specific growth rate is 25.2% and the coefficient of variation of average specific growth rates is 2.3%. Therefore, RMS considers this study as valid.

EC20 values are requested by Regulation 283/2013 but have not been calculated. Given that they are not necessary neither for the risk assessment of glyphosate (that could be performed based on EC50) nor for classification purpose, RMS considers EC20 calculations not necessary for this study.

72h NOErC = 32 mg glyphosate acid/L (nom)
72h ErC10 = 33 mg glyphosate acid/L (nom)
72h ErC50 = 54 mg glyphosate acid/L (nom)

72h NOEbC = 10 mg glyphosate acid/L (nom)
72h EbC10 = 18 mg glyphosate acid/L (nom)
72h EbC50 = 48 mg glyphosate acid/L (nom)

Data point:	CA 8.2.6.1/008
Report author	[redacted]
Report year	1995
Report title	Fresh water algal growth inhibition test with glyphosate
Report No	R481
Document No	-
Guidelines followed in study	No information mentioned in the Monograph.
GLP	Yes
Previous evaluation	According to the applicant: Not accepted in RAR (2015)
Short description of study design and observations:	Toxicity of technical glyphosate (purity >94 %) to aquatic organisms (*Pseudokirchneriella subcapitata*)
Short description of results:	No information mentioned in the Monograph
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available and no information mentioned in the Monograph 2001. However, these data were considered as not acceptable in the Monograph 2001
Reasons why the study report is not available for submission (given by applicant)	The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability:	According to applicant: Invalid (as previously proposed in RAR 2015)

Assessment and conclusion by RMS:
RMS notes that the endpoints from this study (reference R481) were reported but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was requested to the BVL and was provided to RMS.
The endpoint measured in this study (ErC50 = 54 mg glyphosate acid/L and EbC50 = 48 mg glyphosate acid/L) are not critical and therefore the study was not reanalysed by RMS.

Data point	CA 8.2.6.1/009
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Selenastrum capricornutum*
Report No	1092-02-1100-1
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Minor: - Initial nominal cell density of 3×10^3 cells/mL was below the recommended density of $5 \times 10^3 – 10^4$ cells/mL for *P. subcapitata*, however validity criteria were met
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of glyphosate technical on *Pseudokirchneriella subcapitata*, (formerly named *Selenastrum capricornutum*, currently named as *Raphidocelis subcapitata*) were evaluated in a 7-day static toxicity test. After a range-finding test, suspensions of *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations encompassing 10, 18, 32, 56 and 100 mg test item/L. In addition, a control with the test medium (without test substance) was tested.

The test flasks were inoculated with cells from a seven-days-old pre-culture of *Pseudokirchneriella subcapitata* with an initial test cell density of 3000 cells/mL. The test concentrations and the control comprised 3 replicates. The test flasks were placed in the incubator and maintained over several generations for 7 days. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation.

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. On the basis of the mean cell count, the percentage inhibition was determined and the EC_x values calculated using the algal growth curve as determined by inverse estimation least squares linear regression.

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.3% for the lowest test concentration to > 97.6% at or above the nominal test concentration of 18 mg test item/L.

A statistical evaluation (CA 8.2.6.1/010, [redacted] 2020) addressing the calculation of valid 72-h EC10, EC20 and EC50 as well as NOEC values for yield and growth rate was conducted for the algae study 1092-02-1100-1 ([redacted] 1987) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The author concluded that calculated EC_{10}, EC_{20} and EC_{50} values are <10, 10.8, and 27.4 mg a.e./L, respectively for growth rate and < 10.0, 10.3 and 12.1 mg/L, respectively for yield. NOEC for yield and

growth rate were determined to be < 10.0 mg a.e./L. The statistical parameters showed that these values can be considered as reliable and therefore considered for risk assessment.
RMS recalculated the 72h-ErC50 and found an ErC50 of 20.1 mg/L (95% CI : 12 – 34.9 mg/L) wich is considered acceptable for risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6%
Water solubility:	1.2% at 25°C
Vehicle of test material/media:	Vehicle: Dilution water (AAP medium)
Test organism:	
Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration	3×10^3 cells/mL
Source:	In-house culture
Acclimatisation period:	7 days
Environmental conditions:	
Temperature:	24 ± 2°C
Photoperiod:	24 h light
Light intensity:	4306 ± 650 Lux
pH:	7.5 ± 0.1

B. STUDY DESIGN

Experimental dates: 20 April - 27 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with five nominal concentrations (10, 18, 32, 56 and 100 mg test item/L) and three replicates per test item treatment group. Test concentrations were prepared by adding the required volumes of the stock solution to AAP medium in 250 mL volumetric flasks. A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 3000 cells/mL. Flasks were kept in an incubator at a temperature of 24 ± 2°C. Flasks were continuously shaken at 100 oscillations per minute.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Three counts per replicate were made. On the basis of the mean cell count, the percentage inhibition was determined. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation. Samples of test media were taken at test initiation and test termination for analysis of the active

ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations

To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 % inhibition and the associated 95 % confidence intervals were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on nominal concentrations.

Table B.9.2.6.1.1-9: Toxicity of glyphosate technical to *Pseudokirchneriella subcapitata*

Endpoint	Glyphosate technical [mg test item/L]
EC_{50} (7 day)	13.8

Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 10.6, 19.6, 35.2, 58.8 and 104 mg test item/L, corresponding to 106%, 109%, 110%, 105% and 104% of the nominal test concentrations, respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

B. OBSERVATIONS

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.3 % for the lowest test concentration to > 97.6% at or above the nominal test concentration of 18 mg test item/L.

Table B.9.2.6.1.1-10: Percentage growth inhibition of *Pseudokirchneriella subcapitata* exposed to glyphosate for 7 days

Nominal concentrations [mg test item/L]	Control	10	18	32	56	100
Mean number of algae cells on Day 7 [× 1000 cells/mL]	7000	6347	168.333	11.0	9.333	8.333
Mean inhibition (7 days) [%]	-	9.3	97.6	99.8	99.9	99.9

Table B.9.2.6.1.1-11: Mean cell densities and percentage of inhibition of cell growth of *Selenastrum capricornutum* exposed for 72 and 96 hours to glyphosate

	Control	Glyphosate acid [mg test item/L]				
Test parameters	**-**	**10**	**18**	**32**	**56**	**100**
Mean cell densities (0-72 h) (× 10000 cells/mL)	74.1	61.6	2.9	1.3	1.2	1.0
Mean cell densities (0-96 h) (× 10000 cells/mL)	333.3	269.7	3.7	1.17	1.0	0.87
Mean yield inhibition (0-72 h) [%]	-	16.9	96.5	98.6	98.8	99.1
Mean yield inhibition (0-96 h) [%]	-	19.1	98.9	99.7	99.8	99.8
Mean growth rate inhibition (0-72 h) [%]	-	3.3	58.8	72.5	74.8	78.1
Mean growth rate inhibition (0-96 h) [%]	-	3.0	64.2	80.6	82.8	84.9

III. CONCLUSIONS

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10}, EC_{20}, and EC_{50}, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	247
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	0.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤ 7%	1.7%

The biomass in the control cultures increased by a factor of ≥16 (achieved: 247), the coefficient of variance for section specific growth rates was ≤ 35% (achieved: 0.6%) and the coefficient of variance for the whole test period it was ≤ 7% (achieved: 1.7%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC was performed (Positon Paper No. CA 8.2.6.1/010). Recovery of test item concentrations ranged from 100 – 114%. Therefore, results are based on nominal concentrations.

Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC value based on nominal test concentrations

Endpoint (0 – 72 hours)	**Glyphosate technical [mg a.e./L]**	
	Yield	**Growth rate**
EC_{10} (95% CI)	< 10	< 10.0
EC_{20} (95% CI)	10.25 (9.46 – 10.9)	10.8 (< 10.0 – 15.4)
EC_{50} (95% CI)	12.11 (11.4 – 12.8)	27.4 (20.2 – 36.6)
NOEC	< 10.0	< 10.0
LOEC	10.0	10.0

CI = confidence interval

Assessment and conclusion by RMS:
The study is valid. All validity criteria were met according to OECD 201 (2011) guideline. As no algal measurement was done at 24h, RMS adapted the calculation of the mean coefficient of variation for section-by-section between day 0 and day 2 according to OECD 201. RMS found that the biomass in the control cultures increased by a factor of 247, that the mean coefficient of variation for section-by-section specific growth rate is 0.77% and that the coefficient of variation of average specific growth rates is 1.5%.
The light intensity was not in the proposed range of 4440-8880 lux as per the guideline (actual : 4306 ± 650 Lux). This is considered as a minor deviation.
The study was conducted over 7 days, which is considered too long to derive an endpoint given that the exponential growth decreased after 4 days in the control in the study. Nevertheless, raw data allows calculating endpoints for lower durations (48h, 72h and 96h). Endpoints recalculated by applicant in the statistical re-evaluation are for 72h, which seems acceptable to RMS as ECx values at 96h are not recommended in the OECD 201 guideline and in addition, they are expected to be in the same range as the 72h ECx values based on the growth rate inhibition data. The statistical analysis of the endpoints conducted by applicant is accepted by RMS (see study summary and RMS opinion below).

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.6.1/010
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 1092-02-1100-1 on the toxicity of Glyphosate Technical to *Selenastrum capricornutum* under static conditions
Report No	110054-003
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable None
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details
Study number: 1092-02-1100-1
Author: [redacted]
Substance: Glyphosate Technical
Title: The Toxicity of Glyphosate Technical to *Selenastrum capricornutum*
Completion date: 27-Apr-1987

Test guideline(s):	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2) and re-evaluated according to the current test guideline OECD 201 (2011)
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Testing facility:	Malcolm Pirnie, Inc., mite Plains, NY 10602, USA
Sponsor:	Monsanto Agricultural Company, Chesterfield, MO 63198, USA

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC_{10}, EC_{20}, and EC_{50}, and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 1092-02-1100-1 ([redacted] 1987) was statistically evaluated for the effects of glyphosate technical on the organism *Pseudokirchneriella subcapitata*, (formerly named *Selenastrum capricornutum*, currently known as *Raphidocelis subcapitata*). The organisms were exposed for 7 days to the following concentrations of Glyphosate technical: 10, 18, 32, 56 and 100 mg a.s./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on yield and growth rate of the test subjects (EC_{10} EC_{20} and EC_{50}), a non-linear regression analysis was performed with a 3-parametric logistic CDF (Cumulative Distribution Function) model for yield and with probit analysis for growth rate.

NOEC levels were determined by Welsh-t-test After Bonferroni-Holm Correction (one-sided smaller; $p = 0.05$).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Result are provided in the table below:

Validity criteria acc. to OECD 201 (2011)	**Required (0 - 72 h)**	**Obtained (0 - 72 h)**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	247
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	0.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤ 7%	1.7%

Analytical recovery of test item ranged from 100 – 114% of nominal test concentrations. Therefore, results are based on nominal concentrations.

EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

For yield, the parameters for the 3-parameter logistic CDF model are estimated as b0: 73.835; b1: 12.108; b2: 8.330.

For growth rate, the parameters for the probit model are estimated as slope b: 2.08968; Intercept a: -3.00423.

For yield, the statistical parameters are: $F(2, 3) = 1250{,}486$; p(F) = <0.001; R2 = 0.984. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.838.

For growth rate, statistical parameters for goodness of fit test are: Chi2(13) = 1.61163; p(Chi²): 1.000; F(1,13) = 41.449; p(F) <0.001; r²: 0.761 for growth rate.

Based on these values the EC_{10}, EC_{20} and EC_{50} for yield and growth rate calculations should be considered valid.

The obtained EC_{10}, EC_{20} and EC_{50}, and NOEC values for *Raphidocelis subcapitata*, (formerly known as *Selenastrum capricornutum* or *Pseudokirchneriella subcapitata*) are presented in the table below.

Table B.9.2.6.1.1-12: Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC value based on nominal test concentrations

Endpoint (0 – 72 hours)	**Glyphosate technical [mg a.e./L]**	
	Yield	**Growth rate**
EC_{10} (95% CI)	< 10.0	< 10.0
EC_{20} (95% CI)	10.3 (9.46 – 10.9)	10.8 (< 10.0 – 15.4)
EC_{50} (95% CI)	12.1 (11.4 – 12.8)	27.4 (20.2 – 36.6)
NOEC	< 10.0	< 10.0
LOEC	10.0	10.0

CI = confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC_{10}, EC_{20} and EC_{50} values are <10, 10.8, and 27.4 mg a.e./L, respectively for growth rate and < 10.0, 10.3 and 12.1 mg/L, respectively for yield. NOEC for yield and growth rate were determined to be < 10.0 mg a.e./L. The statistical parameters showed that these values can be considered as reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. RMS agrees with ECx calculations. RMS noted that the results indicated more than 50% inhibition of mean growth rate for concentrations greater than 18 mg/L. Thus the 72h-ErC50 was expected to be between 10 and 18 mg/L. After recalculation with RegTox Tool using bootstrap calculations, RMS found a ErC50 of 20.1 mg/L (95% CI : 12 – 34.9 mg/L) wich is considered acceptable for risk assessment.

72h ErC10 < 10 mg glyphosate acid/L (nom)
72h ErC20 = 10.8 mg glyphosate acid/L (nom)
72h ErC50 = 20.1 mg glyphosate acid/L (nom)

72h EyC10 < 10 mg glyphosate acid/L (nom)
72h EyC20 = 10.25 mg glyphosate acid/L (nom)
72h EyC50 = 12.11 mg glyphosate acid/L (nom)

Data point:	CA 8.2.6.1/011
Report author	[redacted]
Report year	1995
Report title	Glyphosate: Algal inhibition test
Report No	710/12
Document No	-
Guidelines followed in study	No information mentioned in the Monograph.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Toxicity of glyphosate acid to aquatic organisms (*Desmodesmus subspicatus*) 72 hours static test.
Short description of results:	NOECb = 25 mg a.s./L NOECr = 25 mg a.s./L E_rC_{50} (24 h) = 60 mg a.s./L E_bC_{50} (72 h) = 46 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.
Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability (RMS)	Supportive. The study report is not available to the applicant. Data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015. Validity cannot be checked. Other valid studies with more sensitive endpoints are available.

Assessment and conclusion by RMS:

RMS notes that the endpoints from this study (reference 95-00535) were reported but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was requested to the BVL but could not be provided to RMS.
For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical.
The endpoint measured in this study is not critical and therefore its absence has no consequence on the risk assessment.

Data point:	CA 8.2.6.1/012
Report author	[redacted]
Report year	1994
Report title	**Unknown**
Report No	XX-93-271
Document No	-
Guidelines followed in study	Information mentioned in the Monograph: The data presented below were generated in accordance with OECD- or equivalent guidelines.
GLP	Yes
Previous evaluation	Yes, accepted in RAR (2015)
Short description of study design and observations:	Acute and chronic toxicity of glyphosate isopropylamin-salt to aquatic organisms (purity 61-65 %) 72 hours static test.
Short description of results:	NOECb = 4.8 mg a.s./L NOECr = 24.0 mg a.s./L E_rC_{50} (72 h) = 166 mg a.s./L E_bC_{50} (72 h) = 72.9 mg a.s./L
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available. However, these data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015.
Reasons why the study report is not available for submission (given by applicant)	The notifier has no access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.
Acceptability/Reliability (RMS)	Supportive. The study report is not available to the applicant. Data were provided in the Monograph 2001 and relied upon in the previous evaluation, RAR 2015. Other valid studies with more sensitive endpoints are available.

Assessment and conclusion by RMS:

The report title indicated by the applicant actually corresponded to an other study that is available and assessed by RMS (*Testing of toxic effects of aminomethylphosphonic acid (AMPA) on the single cell green alga Scenedesmus subspicatus*). It was therefore deleted. The report title of the missing study is unknown but the endpoints provided correspond to the study referenced 95-00554 in RAR 2015 (that is indeed missing within this dossier). The tested item was Glyphosate-IPA salt.

RMS notes that the endpoints from this study were reported but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical.

The endpoint measured in this study (ErC50 (72 h) = 166 mg a.s./L; EbC50 (72 h) = 72.9 mg a.s./L) are not critical and therefore the study was not reanalysed by RMS.

Data point	CA 8.2.6.1/013
Report author	[redacted]
Report year	1993
Report title	Algae growth inhibition test – Test article: "Glyphosate isopropylamine salt"
Report No	80-91-2328-01-93
Document No	-
Guidelines followed in study	OECD Guideline 201(1984) and in compliance with "Hemmung der Zellvermehrung bei Grünalge *Scenedesmus subspicatus* – Verfahrensvorschlag der ad hoc Arbeitsgruppe des Umweltbundesamtes Berlin"
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 47.5%, instead of ≤ 35%, and the coefficient for the whole period was 7.8% instead of ≤ 7%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary

The effects of glyphosate isopropylamine salt on *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) were evaluated in a 72-hour static toxicity test. After a range-finding test *D. subspicatus* were exposed to six nominal concentrations encompassing 1.6, 5.0, 15.8, 50.0, 158 and 500 mg test item/L.

For each concentration, four parallel cultures in 250 ml Erlenmeyer flasks were prepared. The initial cell concentration was 10^4 cells/mL. For the control group, six parallel test vessels were prepared.

After 24, 48, and 72 hours of growth, the numbers of viable cells for each test concentrations and control were determined and the growth inhibition was calculated. At this, concentrations resulting in 50 % inhibition (E_rC_{50}, EbC_{50}), were determined, as well as the NOEC.

The EbC and ErC values were calculated by the mean of dose response curve in regression analysis. The EC50 and EC10 values calculated on the basis of the area under the curve are designated as EbC and the EC values based on the calculation of the growth rate are designated as E_rC.

The 72 h E_rC_{50} for *Desmodesmus subspicatus* was determined to be 241 mg glyphosate isopropylamine salt/L. The 72 h E_bC_{50} for *D. subspicatus* was 41.1 mg glyphosate isopropylamine salt/L. Significant effects of glyphosate isopropylamine salt on the growth of *D. subspicatus* were found at a concentration >15.8 mg test item/L. The NOEC was 15.8 mg test item/L. The validity criteria according to the current guideline OECD 201 were not met. Therefore, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Test item:	Glyphosate isopropylamine salt
Lot No.:	01/06/93
Chemical purity:	61.6%
Physical state:	viscous liquid
Density:	1.23 g/cm3 at 20 °C

Test organism:

Species:	*Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*)
Initial cell concentration:	10^4 cells/mL
Source:	Pflanzenphysiologisches Institut, Göttingen, Germany (Stock No. 8681 SAG)

Environmental conditions:

Temperature:	21 – 23 °C
Photoperiod:	24 h light
Light intensity:	10900 – 11200 lux
Light quality:	Universal white light (8 × 25 W)
pH:	6.69 – 10.59
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 26 July – 29 July 1993

Experimental treatments:
On the basis of the results of a range finding test, the main test was performed with six concentrations, 1.6, 5, 15.8, 50, 158 and 500 mg test item/L.
To maintain the algae in the suspension and to facilitate transfer of CO_2 during the test, the flasks were rotated continuously over the entire test period. For each concentration, four parallel cultures in 250 ml Erlenmeyer flasks were prepared. To each Erlenmeyer flask, 100 mL of the test item preparation were added. The initial cell concentration was 10^4 cells/mL. For the control group, six parallel test vessels were prepared.

Observations:
After 24, 48, and 72 hours of growth, the numbers of viable cells for each test concentrations and control were and the growth inhibition was calculated. At this, the mean value of the cell concentration (converted in log values) was plotted versus percentage growth inhibition to generate dose-response curves for each concentration. The concentrations resulting in 50% inhibition (ErC50, EbC50), were determined, as well as the NOEC.

Statistical calculations:
The area under the growth curves, the percentage inhibition of the cell growth at each test concentration, the average specific growth rate for exponentially growing cultures were calculated according to formulas in OECD 201 (1984). The EC50 and EC10 values calculated on the basis of the area under the curve are designated as E_bC, and the EC values based on the calculation of the growth rate are designated as E_rC. The E_bC and E_rC values on the basis of nominal concentrations were calculated by regression analysis after log transformation of the concentration values.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC10, EbC10, ErC50, EbC50 and NOEC values are given below based on nominal concentrations.

Table B.9.2.6.1.1-13: Toxicity of Glyphosate isopropylamine salt to *Desmodesmus subspicatus*

Endpoint (72 h)	Glyphosate isopropylamine salt [mg test item/L]
ErC10	18.9
EbC10	6.3
ErC50	241
EbC50	41.1
NOEC	15.8

Analytical measurements were performed by HPLC on four representative concentration levels of glyphosate isopropylamine salt, at 15.8 mg test item/L, equivalent to 7.21 mg glyphosate/L, 50 mg test item/L, equivalent to 22.82 mg glyphosate/L, 158 mg test item/L, equivalent to 72.12 mg glyphosate/L and at the highest concentration tested, 500 mg test item/L, equivalent to 228.22 mg glyphosate/L. The analytical results of the determination of glyphosate isopropylamine salt on the basis of glyphosate are given below.

Table B.9.2.6.1.1-14: Measured concentration and recoveries of glyphosate isopropylamine salt based on glyphosate

Nominal concentration		Measured concentration [mg glyphosate/L]		Recovery [%]	
[mg glyphosate isopropylamine salt/L]	[mg glyphosate/L]	0 h	72 h	0 h	72 h
500	228.216	198.901	197.598	87.2	86.6
158	72.116	74.271	72.599	103.0	100.7
50	22.822	25.318	24.479	110.9	107.3
15.8	7.212	7.834	7.607	108.6	105.5

As the measured contents of glyphosate ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

B. OBSERVATIONS

The results of the definite test show that algae growth was completely inhibited at a nominal concentration of 500 mg test item/L. In contrast, no inhibition of the algae growth was found at or below a nominal concentration of 15.8 mg test item/L.

Table B.9.2.6.1.1-15: Percentage inhibition of growth rate, yield and biomass of to *Desmodesmus subspicatus* exposed for 72 hours to glyphosate isopropylamine salt

Glyphosate isopropylamine salt [mg test item/L]	Mean number of algae cells [10000/ml]	Inhibition growth rate (0-72 h) [%]	Inhibition biomass (0-72 h) [%]
Control	119.1	-	-
1.6	107.4	-5.3	-9.5
5	123.9	-15.0	-4.4
15.8	112.2	17.9	12.4
50	26.1	16.9	69.8
158	15.8	34.2	86.3
500	1.9	84.7	96.9

The required minimum of a 16-fold cell multiplication in the control cultures during the test period was achieved.

III. CONCLUSIONS

The 72 h E_rC_{50} for *Desmodesmus subspicatus* was determined to be 241 mg glyphosate isopropylamine salt/L. The 72 h E_bC_{50} for *D. subspicatus* was 41.1 mg glyphosate isopropylamine salt/L. Significant effects of glyphosate isopropylamine salt on the growth of *D. subspicatus* were found at a concentration >15.8 mg test item/L. The NOEC was 15.8 mg test item/L.

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	66.2
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	47.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	7.8%

The biomass in the control cultures increased by a factor of ≥ 16 (actual: 66.2), the coefficient of variance for section specific growth rates exceeded 35% (actual: 47.5%), for the whole test period it exceeded 7% (actual: 7.8%). Because the coefficient of variation for the section specific growth rate was > 35%, and the coefficient for the whole period was > 7%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The study is not valid as the mean coefficient of variation for section-by-section specific growth rates in the control exceeded the trigger of 35% and the coefficient of variation of average specific growth rate during the whole peiod exceeded the trigger of 7%..

Data point:	CA 8.2.6.1/014
Report author	[redacted]
Report year	1990
Report title	Algal growth inhibition test with compound glyphosate TCN
Report No	1-7-46-90
Document No	-
Guidelines followed in study	OECD 201
GLP	Yes
Previous evaluation	Applicant : Not accepted in RAR (2015) RMS : Study not reported in RAR 2015
Short description of study design and observations:	The toxicity of Glyphosate TCN to *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) was determined in a 96 - hour static test. The test incorporated 5 nominal concentrations at 20, 50 100, 200 and 400 mg a.s./L (Glyphosate TCN: sample No. 16/03/90 with 95% purity) and an untreated control. The test comprised three replicate cultures of each test concentration and the control. The initial nominal cell density was 1.00×10^4 cells/mL. The cell densities were determined microscopically with the help of the Neubauer counting chamber. Cell numbers were counted at test start, after 72 and 96 hours. The pH-values and O2 values were determined in the test media at the beginning and at the end of the test. The room temperature was 22 ± 2°C and light intensity was approx. 8000 lux. The algae were illuminate continuously with fluorescent lamp (Universalweiß Typ L 25, Osram)
Short description of results:	**Glyphosate TCN (mg a.s./L)** / **Inhibition in algal growth (%)** Control / - 20 / 0 50 / 6.7 100 / 33.3 200 / 55.4 400 / 84.6 **Endpoints (96 h)** / **Glyphosate TCN (mg a.s./L)** LC10 / 56 ± 26 LC50 / 136 ± 64 There were no differences in parameters oxygen and temperature between the test item treatments and the control. The pH values decreased very clearly with increasing dosage. At 400 mg a.s./L 50% of the cells were damaged. This was not observed in the other test item concentrations.
Reasons for why the study is not considered relevant/reliable or not considered as key study:	Applicant: The study design is not in line anymore with the current guideline OECD 201 requirements (*eg*. control biomass and section specific growth rates were not determined, no analytical measurement performed). The validity criteria according to the current guideline could not be concluded.

	Therefore, no consistent conclusions could be drawn from the study. The study is considered as not relevant according to various shortcomings. RMS: please refer to assessment and conclusion by RMS below
Reasons why the study report is not available for submission (given by applicant)	Applicant: The notifier has not access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL. RMS : Study report in caddy. See assessment and conclusion of RMS below
Acceptability/Reliability (RMS)	Invalid.

Assessment and conclusion by RMS:
The study report has been checked by RMS and considers this study not reliable for risk assessment. Indeed, only mean values of the number of cells counted are available and therefore validity criteria could not be checked. Moreover, analytical measurements of the active substance were not conducted during the study and raw data are not available.

Data point	CA 8.2.6.1/015
Report author	[redacted]
Report year	1990
Report title	Acute Toxicity of Glyphosate to *Scenedesmus subspicatus* (OECD – Algae Growth Inhibition Test)
Report No	250773
Document No	
Guidelines followed in study	OECD Guideline 201 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 101.6%, instead of ≤ 35%
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary
The effects of glyphosate on *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) were evaluated in a 96-hour static toxicity test. Based on the results of a range finding test, *Desmodesmus subspicatus* were exposed to five nominal concentrations encompassing 1.6, 8.0, 40, 200 and 1000 mg test item/L and a control.

For each test concentration and control treatment three replicates with 30 mL test solution and an initial cell density of 104 cells/mL were prepared in 50 mL Erlenmeyer flasks. Additionally, for the highest test concentration one replicate without algae was provided. The culture vessels were incubated in a shaking water bath at 24°C for 96 hours.

After 24, 48, 72 and 96 hours, the number of algae was estimated microscopically after 24 and 48 hours and after 72 and 96 hours by spectrophotometer.

Test item concentrations were verified by HPLC in the 1.6, 40 and 1000 mg test item/L test item treatments and the 1000 mg/L stability control at the beginning and the end of the test (after 96 hours). During the test period test item concentrations were in the range from 56.9 to 66.6% of the nominal values. Therefore, all reported results are related to mean measured concentrations of the test item.

Glyphosate inhibited cell growth of the fresh water algae *Desmodesmus subspicatus* after 72 hours at mean measured concentrations of 200 and 1000 mg test item/L and after 96 hours at mean measured concentrations of 8.0, 40, 200 and 1000 mg test item/L.

The 72 hours EbC_{50} for *Desmodesmus subspicatus* exposed to glyphosate was 326.9 mg/L (300.2 – 354.3 mg test item/L), the 96 hours E_bC_{50} was 117.8 mg/L (107.3 - 129.5 mg test item/L). The NOEC and LOEC for *D. subspicatus* after 96 hours of exposure were 40 and 200 mg test item/L, respectively.

Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate
Description:	Solid
Lot/Batch #:	198-SI-22-1
Purity:	98.7%
Solubility:	Aqueous: 12000 mg/L at 25 °C
Vehicle of test material/media and positive control:	Vehicle: Test medium Positive control: Potassium dichromate (K2Cr2O7)

Test organism:

Species:	Algae (*Desmodesmus subspicatus)*
Initial cell concentration	10^4 cells/mL
Source:	Umweltbundesamt, Berlin, Germany

Environmental conditions:

Temperature:	24.0°C
Photoperiod:	24 h light
Light intensity	8000 lux
pH:	7.7 (adjusted at test start), 6.3 (control), 7.3 (mean of all test concentrations)

B. STUDY DESIGN

Experimental dates: 9 to 13 October 1989

Experimental treatments
Based on the results of a range-finding test the definitive study encompassed five nominal concentrations: 1.6, 8.0, 40, 200 and 1000 mg test item/L. In addition, algae (*Desmodesmus subspicatus)*

were exposed to test medium without test substance or other additives (control).
The culture vessels were incubated on a shaking plate in a water bath at 24 °C for 96 hours. During incubation, the algal cells were kept in suspension by continuous shaking. For each concentration and the control, three replicates were prepared in 50 ml Erlenmeyer flasks. To each test vessel, 30 mL of the test item preparation were added with an initial cell density adjusted to 104 cells/mL. Additionally, for the highest test concentration one replicate without algae was provided.

Observations
After 24 and 48 hours, the number of algae was estimated microscopically and spectrophotometrically after 72 and 96 hours. The concentrations resulting in 50 % reduction of growth rate (E_bC_{50}), 100 % reduction of growth rate (E_bC_{100}) and no growth rate reduction (E_bC_0) were determined as area under the growth curve. The pH-values of the test solutions were adjusted at test initiation and measured at test termination. Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis, using duplicate samples of 5 mL taken from the low (1.6 mg/L), medium (40 mg/L) and high (1000 mg/L) test concentration at test termination. From the additional test vessel containing 1000 mg/L and no algae samples of 100 mL and 10 mL were taken after 0 and 96 hours.

Statistical calculations
Inhibition of cell growth was determined from the area under the growth curve. The NOEC and LOEC after 96 hours were statistically determined with the Dunnett´s test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The E_bC_{50} (0 - 72, 0 - 96 hours), NOEC and LOEC values are given below based on nominal concentrations.

Table B.9.2.6.1-16: Toxicity of glyphosate to *Desmodesmus subspicatus*

Endpoint	Glyphosate [mg test item/L]
0 - 72 hours E_bC_{50} (95 % CI)	326.9 (300.2 - 354.3)
0 - 96 hours E_bC_{50} (95 % CI)	117.8 (107.3 - 129.5)
NOE_bC	40
LOE_bC	200

CL = confidence limit

Analytical control measurements were performed in the test solutions with nominal values of 1.6, 40 and 1000 mg test item/ and at 1000 mg test item/L without algae. At test initiation and test termination the test concentrations were in a range of 56.9 to 66.6% of nominal. In the 1000 mg/L stability test the concentration was 117.3% of nominal at test initiation and 92.9% of nominal at test termination.
As the mean measured content of the test item was not in the range between 80 and 120% of nominal, the endpoints are given as nominal concentrations.
Reference item: The 96-hour E_bC_{50} was 1.514 mg/L (95% CI: 1.488 – 1.542 mg/L). These results were in agreement with what was expected on the basis of historical data.

B. OBSERVATIONS

Glyphosate inhibited cell growth of the fresh water algae *Desmodesmus subspicatus* after 72 hours at test concentrations of 200 and 1000 mg test item/L and after 96 hours at test concentrations of 8.0, 40, 200 and 1000 mg test item/L.

Table B.9.2.6.1.1-17: Mean cell densities and percentage of inhibition of cell growth *of Desmodesmus subspicatus* exposed for 72 and 96 hours to glyphosate

	Control	Glyphosate [mg test item/L]				
Test parameters	**-**	**1.6**	**8.0**	**40**	**200**	**1000**
Mean cell densities (0 - 72 h) (× 10000 cells/mL)	35.6	38.0	32.8	36.7	18.2	6.5
Mean cell densities (0 - 96 h) (× 10000 cells/mL)	363.7	348.4	291.5	311.2	107.4	0
Cell growth inhibition (0 - 72 h) [%]	-	-2.2	12.1	8.2	62.0	94.6
Cell growth inhibition (0 - 96 h) [%]	-	-21.3	-12.4	-8.5	36.6	78.9

III. CONCLUSIONS

The 72 hours E_bC_{50} for *Desmodesmus subspicatus* exposed to glyphosate was 326.9 mg/L (300.2 – 354.3 mg test item/L), the 96 hours E_bC_{50} was 117.8 mg/L (107.3 - 129.5 mg test item/L). The NOEC and LOEC for *D. subspicatus* after 96 hours of exposure were 40 and 200 mg test item/L, respectively.

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	**Required (0 - 72 h)**	**Obtained (0 - 72 h)**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	35.6
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	101.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	4.9%

The biomass in the control cultures increased by a factor of ≥16 (actual: 35.6), the coefficient of variance for section specific growth rates exceeded 35% (actual: 101.6%), for the whole test period it was ≤ 7% (actual: 4.9%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The study is not valid as the mean coefficient of variation for section-by-section specific growth rates in the control exceeded the trigger of 35%.

Data point	CA 8.2.6.1/016
Report author	[redacted]
Report year	1998
Report title	Fresh Water Algal Growth Inhibition Test with (Aminomethyl)Phosphonic Acid
Report No	232458
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Applicant: Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 58.5% instead of ≤35% RMS recalculated CV section-by-section considering the recommendations of EFSA Supporting publication 2015:EN-924 (Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology). Re-calculated CV is lower than 35%.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes, conducted under GLP officially recognised testing facilities
Acceptability/Reliability (RMS)	Applicant: Invalid (however, study is used for risk assessment, as this is the most reliable algae study with AMPA) RMS : Valid

Summary

The effects of (Aminomethyl)phosphonic acid (AMPA) on *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*, currently known as *Raphidocelis subcapitata*) were evaluated in a 72-hour static toxicity test. After a range-finding test *Pseudokirchneriella subcapitata* were exposed to five nominal concentrations encompassing 10, 22, 46, 100 and 220 mg test item/L and a blank control.

For each test concentration and the control group, three (test concentrations) or six (control) replicates with 50 mL test solution and an initial cell density of 10^4 cells/mL were prepared in 100 mL vessels. The culture vessels were incubated on a shaking plate for 72 h. After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements.

The concentrations resulting in 50% reduction of growth rate (ErC50) and 50% inhibition of cell growth (EbC50) were determined, as well as the associated NOEC values.

Results showed that the cell densities were comparable to those of the control at nominal concentrations up to 46 mg test item/L, while cell densities at 100 mg test item/L and 220 mg test item/L were increasingly reduced. At 220 mg test item/L almost no increase in cell densities were observed during the test period.

Statistically significant inhibition of cell growth was found at test concentrations of 100 mg test item/L and higher.

Growth rates were in the range of the control at concentrations from 10 to 46 mg test item/L during the 72-hour test period, whereas the growth rate of algae exposed to 100 and 220 mg test item/L were

increasingly reduced. Statistically significant reduction of growth rate was found at test concentrations of 100 mg/L and higher.

A statistical evaluation (CA 8.2.6.1/017, [redacted] 2020) addressing the calculation of valid 72-h EC10 EC20 and EC50 as well as NOEC values was conducted for the algae study 232458 [redacted] 1998) to fulfil the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011). The validity criteria according to the current guideline OECD 201 were met for increase of biomass and for coefficient of variation of average specific growth rates in the controls. However, mean coefficient of variation for section-by-section specific growth rate was 58.5% and exceeds the required 35%.
As this is the only study currently available for algae exposed to AMPA, the data was further analysed to obtain the required effect concentrations.
The calculated EC10, EC20 and EC50 values are 58.2, 72.5 and 110 mg/L for yield, respectively and 92.8, 119 and 191 mg/L, respectively for growth rate. The statistical parameters presented showed that these values can be considered reliable/valid and therefore considered for risk assessment.
The author concluded that NOEC for yield and growth rate were 100 mg/L.
RMS concluded that as 35% cell growth inhibition was measured at 100 mg AMPA/L, the NOEyC was 46 mg/L.

The applicant calculated that the coefficient of variation for the section specific growth rate was > 35%, and therefore considered that the validity criteria according to the current guideline OECD 201 were not met. Therefore, the applicant considered that this study is not valid for risk assessment purposes.
RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline. Therefore, RMS considers this study as valid.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	(Aminomethyl)phosphonic acid (AMPA)
Description:	White powder
Lot/Batch #:	A010047101
Purity:	99 %
Vehicle of test material/media and positive control:	Vehicle: Dilution water (ISO-medium) Positive control: Potassium dichromate (K2Cr2O7)

Test organism:

Species:	*Pseudokirchneriella subcapitata*, strain: CCAP 278/4
Initial cell concentration:	1×10^4 cells/mL
Source:	In-house culture
Acclimatisation period:	4 days

Environmental conditions:

Temperature:	22.5 – 23.0°C
Photoperiod:	24 h light
Light intensity:	6000 - 7500 lux
Light quality:	TLD-lamps of 18 Watt
pH:	Blank-control (0 – 72 h): 8.5

10 mg/L (0 – 72 h): 7.7 – 8.0
22 mg/L (0 – 72 h): 7.5 – 8.0
46 mg/L (0 – 72 h): 7.1 – 7.8
100 mg/L (0 – 72 h): 6.2 – 7.0
220 mg/L (0 – 72 h): 6.0 – 6.8

Hardness: 24 mg CaCO3/L

B. STUDY DESIGN

Experimental dates of work: 19 May to 29 May 1998

Experimental treatments
Prior to the main test, a range-finding test was performed with concentrations of 0.1, 1, 10 and 100 mg test item/L. On the basis of these preliminary test results, the main test was performed with five concentrations: 10, 22, 46, 100 and 220 mg test item/L. In addition, algae were exposed to test medium without test substance or other additives (blank control). The test solutions were prepared using ISO-medium.

The culture vessels were incubated on a shaking plate over several generations for 72 h. For each concentration, three parallel cultures were prepared in 100 ml all-glass vessels. To each test vessel, 50 mL of the test item preparation were added, with an initial cell density adjusted to 104 cells/mL. Additionally, for the highest test concentration one replicate without algae was provided. For the control group, six parallel test vessels were prepared.

Observations
After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements and a linear calibration curve relating extinction and cell density.
The concentrations resulting in 50% reduction of growth rate (E_rC_{50}) and 50 % inhibition of cell growth (E_bC_{50}) were determined, as well as the associated NOEC values.
The pH values of the test solutions were measured at test initiation and test termination. Temperature was controlled daily in a temperature-control vessel.

Analytical control measurements of the actual concentration of the test item were performed by HPLC analysis using samples taken from three representative concentrations, 10, 46 and 220 mg test item/L.

Statistical calculations
The calculation of the EC_{50} values was based on linear regression analysis of the percentages of growth inhibition and the percentages of growth rate reduction versus the logarithms of the corresponding nominal concentrations of the test substance.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC50, EbC50 and NOEC values are given below, based on nominal concentrations.

Table B.9.2.6.1.1-18: Toxicity of AMPA to *Pseudokirchneriella subcapitata*

Endpoint (0 – 72 hours)	AMPA [mg test item/L]
E_rC_{50} (95% CI)	200 (98 - 410)
E_bC_{50} (95% CI)	110 (72 - 180)
E_rC_{10} (95% CI)	68 (34 - 140)
E_bC_{10} (95% CI)	53 (33 - 86)
NOE_rC	46
NOE_bC	46

CI = confidence interval

Analytical data: Analytical control measurements were performed on three representative concentrations. At test initiation, 99%, 100% and 102% of the test item were recovered for the nominal concentrations of 10, 46 and 220 mg test item/L, respectively. At test termination, 98%, 98% and 96% of the test item were recovered for the nominal concentrations of 10, 46 and 220 mg test item/L, respectively.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Reference item: The 72-hour E_bC_{50} was 1.3 mg reference item/L (95 % CI: 0.34 - 4.6 mg reference item/L), the 72-hour E_rC_{50} was 1.7 mg reference item/L (95 % CI: 1.1 - 2.8 mg reference item/L).

B. OBSERVATIONS

Mean cell densities: Cell densities were comparable to blank at nominal concentrations up to 46 mg test item/L while cell densities at 100 mg test item/L and 220 mg test item/L were increasingly reduced. At 220 mg test item/L almost no increase in cell densities were observed during the 72 hour test period.

Inhibition of cell growth: Inhibition of cell growth increased with increasing concentration of AMPA from a nominal concentration of 22 mg test item/L upwards. Statistically significant inhibition of cell growth was found at test concentrations of 100 mg test item/L and higher.

Reduction of growth rate: Growth rates were in the range of the controls at the concentrations from 10 to 46 mg test item/L during the 72-hour test period, whereas the growth rate of algae exposed to 100 and 220 mg test item/L were increasingly reduced. Statistically significant reduction of growth rate was found at test concentrations of 100 mg test item/L and higher.

Table B.9.2.6.1.1-19: Percentage reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to AMPA

Test parameters (0 – 72 hours)	Control	AMPA [mg test item/L]				
	-	10	22	46	100	220
Mean cell densities (× 10000 cells/mL)	67.8	73.0	67.6	64.5	41.5	5.4
Cell growth rate reduction [%]		-1.7	0.1	1.2	12.0	59.8
Cell growth inhibition [%]		-3.5	3.0	6.6	35.4	87.8

In the controls, cell density increased by an average factor of > 16 within 3 days. Analysis of samples taken from the solution without algae showed that the actual exposure concentration remained above 80% relative to the initial concentration. Further, all test conditions remained within the ranges prescribed by the protocol.

III. CONCLUSION

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	67.9
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	58.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤ 7%	0.6%

The biomass in the control cultures increased by a factor of ≥16 (actual: 67.9), the coefficient of variance for section specific growth rates exceeded 35% (actual: 58.5%), for the whole test period it was ≤ 7% (actual: 0.6%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.
However, due to the more severe shortcomings of the algae study with *Desmodesmus* exposed to AMPA (CA 8.2.6.1/018, [redacted] 1994), this study is used in risk assessment.

A statistical re-evaluation addressing EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC was performed (Positon Paper No. 110054-004).

Since analytical recoveries of the test item ranged from 96 to 102%, results are based on nominal test concentrations.

Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	AMPA [mg/L]	
	Yield	Growth rate
EC_{10} (95% CI)	58.2 (45.3 – 74.8)	92.8 (84.6– 102)
EC_{20} (95% CI)	72.5 (57.4– 91.8)	119 (109– 130)
EC_{50} (95% CI)	110 (82.2– 147)	191 (171 – 213)
NOEC	100	100
LOEC	220	220

Assessment and conclusion by RMS:
RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline. The biomass in the control cultures increased by a factor of 67.9, the mean coefficient of variation for section-by-section specific growth rate is 29.0% and the coefficient of variation of average specific growth rates is 1.0%. Therefore, RMS considers this study as valid.

Given that 35% cell growth inhibition was measured at 100 mg AMPA/L, RMS considers the NOEyC to be 46 mg/L.

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.6.1/017
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 232458 on the toxicity of (Aminomethyl) phosphonic acid (AMPA) to *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) under static conditions
Report No	110054-004
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number:	232458
Author:	[redacted]
Substance:	(Aminomethyl) phosphonic acid (AMPA)
Title:	Fresh Water Algal Growth Inhibition Test with (Aminomethyl)Phosphonic Acid
Completion date:	29 June 1998
Test guideline(s):	OECD Guideline No. 201 (1984) EEC Directive 92/69, Part C-3 (1992) ISO International Standard 8692 (1989)
GLP:	Yes
Testing facility:	NOTOX B.V., DD 's-Hertogenbosch, The Netherlands
Sponsor:	AgriChem BV, AG OOSTERHOUT, The Netherlands

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC10, EC20, and EC50, and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 232458 ([redacted] 1998) was statistically evaluated for the effects of (Aminomethyl) phosphonic acid (AMPA) on the organism *Pseudokirchneriella subcapitata*, strain: CCAP 278/4 (currently known as *Raphidocelis subcapitata*). The organisms were exposed for 72 h to the following concentrations of (Aminomethyl) phosphonic acid (AMPA): 10, 22, 46, 100 and 220 mg test item/L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects (EC10, EC20 and EC50), the 3-parametric normal CDF (Cumulative Distribution Function) model was used for growth rate and yield.

NOEC for growth rate and yield was determined by Welsh-t-test After Bonferroni-Holm Correction (one-sided smaller, $p = 0.05$).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met for increase of biomass and for coefficient of variation of average specific growth rates in the controls. However, mean coefficient of variation for section-by-section specific growth rate was 58.5% and exceeds the required 35%.
Results are provided in the table below:

Table B.9.2.6.1.1-20: Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	68
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	58.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤ 7%	0.6%

As this is the only study currently available for algae exposed to AMPA, the data was further analysed to obtain the required effect concentrations.

The mean measured content of the test item always ranged between 80 and 120% of nominal, therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

For yield, the parameters for the 3-parameter normal CDF model are estimated as b0: 68.412; b1: 1.765; b2: 0.216.
For growth rate, the parameters for the 3-parameter normal CDF model are estimated as b0: 1.411; b1: 1.968; b2: 0.244.

For yield, the statistical parameters are: F (2, 3) = 225.575; p(F) = <0.001; R2 = 0.939. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.396.
For growth rate, the statistical parameters are: F (2, 3) = 901.363; p(F) = <0.001; R2 = 0.990. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.637.
Based on these values the EC10, EC20 and EC50 for yield and growth rate calculations should be considered valid.

The obtained EC10, EC20 and EC50 values for *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) are presented in the table below.

Table B.9.2.6.1.1-21: Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	**AMPA [mg test item/L]**	
	Yield	**Growth rate**
EC10 (95% CI)	58.2 (45.3 – 74.8)	92.8 (84.6– 102)
EC20 (95% CI)	72.5 (57.4 – 91.8)	119 (109– 130)
EC50 (95% CI)	110 (82.2 – 147)	191 (171 – 213)
NOEC	100	100
LOEC	220	220

CI = confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met for increase of biomass and for coefficient of variation of average specific growth rates in the controls. However, mean coefficient of variation for section-by-section specific growth rate was 58.5% and exceeds the required 35%.
As this is the only study currently available for algae exposed to AMPA, the data was further analysed to obtain the required effect concentrations.

The calculated EC10, EC20 and EC50 values are 58.2, 72.5 and 110 mg/L for yield, respectively and 92.8, 119 and 191 mg/L, respectively for growth rate. NOEC for yield and growth rate were determined to be 100 mg/L.
The statistical parameters presented showed that these values can be considered reliable/valid and therefore considered for risk assessment.

Assessment and conclusion by RMS:
As stated above, RMS checked the validity criteria and found that all validity criteria were met according to the OECD 201 (2011) guideline and considers the study valid. The statistical analysis is considered valid. RMS agrees with ECx calculations.

72h NOErC = 100 mg AMPA/L (nom)
72h ErC10 = 92.8 mg AMPA/L (nom)
72h ErC20 = 119 mg AMPA/L (nom)
72h ErC50 = 191 mg AMPA/L (nom)

72h NOEyC = 46 mg AMPA/L (nom)
72h EyC10 = 58.2 mg AMPA/L (nom)
72h EyC20 = 72.5 mg AMPA/L (nom)
72h EyC50 = 110 mg AMPA/L (nom)

Data point:	CA 8.2.6.1/018
Report author	[redacted]
Report year	1994
Report title	Testing of toxic effects of aminomethyl phosphonic acid (AMPA) on the single cell green alga *Scenedesmus subspicatus*
Report No	IFU93006/01-Ss
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - Raw data is provided as optical density, however a correlation with biomass is not provided. - Test was conducted in three runs (not replicates). No replicates for each concentration. - In the 2nd and 3rd run, a test substance was used not originally purchased from sponsor, rendering lower absolute growth densities. - Control biomass was not determined and section specific growth rates are not reproducible. - The measured concentrations of AMPA were reported only for one test concentration at the start and at the end of the test.
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Invalid

Summary

The toxicity of AMPA to the green algae *Desmodesmus subspicatus* (formerly known as *Scenedesmus subspicatus*) was determined in a 72-hour, static test. The test incorporated six nominal concentrations of AMPA (0.192, 0.96, 4.8, 24, 120, and 600 mg a.s./L) and a dilution water control without test item. The test was performed in 3 replicates per test concentration and control. At the start of the test, 50 mL test solutions (or test medium without AMPA for the controls) was inoculated with 10^4 algae cells/mL.

The culture vessels were incubated at 23±2°C under continuous illumination for 72 h. The cell number was determined by photometric measurements at 0, 15, 24, 39, 48, 63, and 72 hours of exposure. The pH-values were determined in the test media at the beginning and at the end of the test.

The nominal concentration in the analysed dilution step was 0.96 mg AMPA/L; the analytical values were 0.99 mg /L at the start of the test and 1.06 mg/L at the end of the test. For that reason AMPA can be regarded as stable under test conditions. Due to various deviations from the current OECD 201 guideline, this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item::	Aminomethyl phosphonic acid (AMPA)	
Description:	not stated	
Lot/Batch #:	A)	PIT-8912-1385A
	B)	09203L7
Purity:	A)	99.1%
	B)	99%
Vehicle of test material/media:	Cell growth medium	

Test organism:

Species:	Algae *Desmodesmus subspicatus* CHODAT
Initial cell concentration:	10^4 cells/mL
Source:	Collection of algae cultures, Pflanzenphysiologisches Institut der Universitaet, 37073 Goettingen, Germany
Acclimatisation period:	3 days

Environmental conditions:

Temperature:	23 ± 2 °C (no measured data reported)
Photoperiod:	Continuous illumination
Light intensity:	approximately 8000 lux
pH:	4.32 – 6.39 at the start of the test
	4.34 – 7.34 at the end of the test

B. STUDY DESIGN

Experimental dates: 5 November – 10 December 1993

Experimental treatments
The toxicity of AMPA to the green algae *Desmodesmus subspicatus* was determined in a 72-hour, static test. The test incorporated six nominal concentrations of AMPA (0.192, 0.96, 4.8, 24, 120, and 600 mg test item/L) and a dilution water control without test item. The six test concentrations were prepared by appropriate dilutions of a stock solution. The test was performed in three runs per test concentration and control. At the start of the test, 50 mL test solution (or test medium without AMPA for the controls) was inoculated with 10^4 algae cells/mL. The culture vessels were incubated at 23 ± 2 °C under continuous illumination for 72 hours.

Observations
The cell number was determined by photometric measurements at 0, 15, 24, 39, 48, 63, and 72 hours of exposure. The pH-values were determined in the test media at the beginning and at the end of the test.

Statistical calculations
Graphical determination of endpoints.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC50, EbC50 and NOEC values are given below, based on nominal concentrations.

Table B.9.2.6.1.1-22: Toxicity of AMPA to *Desmodesmus subspicatus* (nominal values)

Endpoints (72 hours)	AMPA [mg test item/L]
NOErC	8.3
ErC10	18.5
ErC50	452
NOEbC	7.9
EbC10	12.9
EbC50	89.8

The nominal concentration in the analysed dilution step was 0.96 mg AMPA/L; the analytical values were 0.99 mg AMPA/L at the start of the test and 1.06 mg/L at the end of the test. For that reason, AMPA can be regarded as stable under test conditions.

B. OBSERVATIONS

AMPA inhibited cell growth of the fresh water algae *Desmodesmus subspicatus* after 72 hours within a test item concentration of 0.1 to 600 mg test item./L (nominal).

Table B.9.2.6.1.1-23: Calculation of the percentage of inhibition for the determination of the EbC value (0-72 h)

Nominal concentration (mg AMPA/L)	Parallel 1		Parallel 2		Parallel 3	
	Area (A)	% inhibition	Area (A)	% inhibition	Area (A)	% inhibition
Control	0.9085	0	0.478	0	0.495	0
0.192	0.980	-7.87*	0.506	-5.85*	0.546	-10.3*
0.96	1.0415	-14.63*	0.5565	-16.42*	0.598	-20.8*
4.8	0.9875	-8.69*	0.515	-7.74*	0.513	-3.63*
24	0.897	1.26	0.446	6.69	0.444	10.3
120	0.6725	25.97*	0.094	80.33	0.116	76.65
600	0.231	74.5	0.080	83.26	0.092	81.41

* Not taken for the calculation

Table B.9.2.6.1.1-24: Calculation of the percentage of inhibition for the determination of the ErC value (0-72 h)

	Parallel 1		Parallel 2		Parallel 3	
Nominal concentration (mg AMPA/L)	**µ (1/h)**	**% inhibition**	**µ (1/h)**	**% inhibition**	**µ (1/h)**	**% inhibition**
Control	0.0871	0	0.0890	0	0.0894	0
0.192	0.0823	5.51*	0.0702	21.12*	0.0810	9.39*
0.96	0.0742	14.81*	0.0712	20.00*	0.0827	7.49*
4.8	0.0785	9.87*	0.0700	21.34*	0.0742	17.00*
24	0.0762	12.51	0.0605	32.02*	0.0716	19.91
120	0.0717	17.68	0.0598	32.80	0.0609	31.88
600	0.0377	56.71	0.0349	60.78	0.0429	52.01

* Not taken for the calculation

III. CONCLUSION

The 72 h EbC50 for *Desmodesmus subspicatus* exposed to AMPA was 89.8 mg test item/L (nominal). The 72 h ErC50 was 452 mg test item/L (nominal).

Assessment and conclusion by applicant:

The study shows various deficiencies.

- Raw data is provided as optical density, however a correlation with biomass is not provided.
- Test was conducted in three runs instead of simultaneous replication of each test concentration.
- For the 2nd and 3rd run, a test substance was used with a different source and lot number compared to the first run, rendering lower absolute growth densities.
- Control biomass was not determined and section specific growth rates are not reproducible.
- The measured concentrations of AMPA were reported only for one test concentration at the start and at the end of the test

Therefore, the study is not considered valid. However, an additional study with AMPA is available.

Assessment and conclusion by RMS:

Validity criteria, biomass and growth rates could not be checked as only optical densities measured by spectrophotometry were presented in raw data and no calibration curve of the relationship between optical density and cell density is available. In addition, three different runs were conducted instead of one with replicates. As validity criteria and effects on biomass and growth rates could not be checked, RMS considers this study not reliable for risk assessment.

Data point:	CA 8.2.6.1/019
Report author	[redacted]
Report year	2011
Report title	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*)
Report No	139A-396A
Document No	-
Guidelines followed in study	OECD Guideline 201 (2006) EU Directive 92/69/EEC, Method C.3. (1992)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
The effects of HMPA on *Pseudokirchneriella subcapitata* were evaluated in a 72-hour static toxicity test. *P. subcapitata* were exposed to five nominal concentrations encompassing 7.5, 15, 30, 60 and 120 mg HMPA/L, and the measured concentrations were 7.3, 14, 29, 60 and 115 mg HMPA/L respectively.
For each concentration, three parallel cultures in 250 ml Erlenmeyer flasks were prepared. The initial cell concentration was 1 x 10^4 cells/mL. For the control group, six parallel test vessels were prepared. After 24, 48, and 72 hours of growth, the numbers of viable cells for each test concentrations and control were determined and the growth inhibition was calculated. Exposure concentrations resulting in 50% inhibition (ErC50, EC50), were determined, as well as the NOAEC. EC50, ErC50 and the corresponding 95% confidence limits for each 24-hour exposure interval were calculated by non-linear regression.
The results of main test showed that the algal growth was not inhibited at the measured test item concentrations of 7.3, 14, 29 and 60 mg HMPA/L, and was inhibited slightly at the measured test item concentration of 115 mg HMPA/L.

The author concluded that 72 h-ErC50 and EC50 for *P. subcapitata* exposed to HMPA was determined both >115 mg HMPA/L and that the NOAEC was 60 mg HMPA/L.
The applicant calculated EyC10 = 57.8 mg/l and ErC10 > 120 mg/L (CA 8.2.6.1/020, [redacted] 2020)
RMS proposed to base the endpoints on nominal concentrations as they have been satisfactorily maintained. For growth rate, 72h NOErC, ErC10 and ErC50 are 60, >120 and >120 mg HMPA/L (nom), respectively. For yield, 72h NOEyC, EyC10 and EyC50 are 60, 57.8 and > 120 mg HMPA/L (nom).

The validity criteria according to the current guideline OECD 201 were met. Therefore, this study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:

Identification:	Hydroxymethyl phosphonic acid (HMPA)
Lot No.:	GLP-1003-20448-A
Chemical purity:	97 %
Physical state:	White powder
Storage condition:	Ambient desiccated
Expiration date:	30 April 2012

Test organism:

Species:	*Pseudokirchneriella subcapitata*
Initial cell concentration:	10^4 cells/mL
Source:	in-house culture, started from University of Toronto Culture Collection

Environmental conditions:

Temperature:	23.0 – 24.8°C
Photoperiod:	24 h light
Light intensity:	6030 – 7040 lux
Light quality:	cool-white fluorescent lighting
pH:	7.0 – 7.2 (test start); 7.5 – 9.3 (test termination)
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 13 June - 16 June 2010

Experimental treatments

Three replicate cultures per test concentration of *P. subcapitata* (initial cell density in each chamber was 1×10^4 cells/mL) were exposed for 72 hours to nominal concentrations of 7.5, 15, 30, 60, and 120 mg HMPA/L. A negative control group with six replicate cultures was held under the same environmental conditions concurrently.

A primary stock solution with a nominal concentration of 120 mg HMPA/L was prepared, and the pH of mixed sufficiently stock solution was determined as 3.0. The pH of the stock solution was adjusted to 7.0 ± 0.1 with 0.1 N NaOH, then another four test solutions with the nominal concentrations of 7.5, 15, 30 and 60 HMPA/L were prepared through proportionally diluting of stirred stock solution.

Observations

Test medium samples were collected from each biological replicate of the treatment and control group for the determination of algal cell densities. Samples were collected at approximately 24-hour intervals during the 72-hour exposure and were held for a maximum of two days under dark, refrigerated conditions sufficient to inhibit growth until cell counts could be performed. Cell counts. Prior to conducting cell counts, the linearity of the instrument response was determined at settings previously established for *P. subcapitata*.

Samples of test solution were collected from each of the replicates per treatment and control group at the end of the test. These samples were pooled within their respective treatments, and subsamples were removed and examined microscopically for atypical cell morphology (e.g., changes in cell shape, size or color). Cells in the replicate test chambers also were assessed for aggregations or flocculation of cells, and adherence of the cells to the test chamber.
Samples of the test solutions were collected at approximately 0 and 72 hours to measure concentrations of the test substance. At test initiation, samples were collected for each treatment and control group prior to distribution of test solution into test chambers. At 72 hours, samples were collected from the pooled biological replicates from each respective treatment and control group.

The temperature was recorded twice daily during the test using a liquid-in-glass thermometer. Light intensity was measured at test initiation. The pH of the medium in each treatment and control group was measured at test initiation and at test termination

Statistical calculations
Cell densities, growth rates and percent inhibition values were calculated according to formulas in OECD 201 (2006) using SAS System for Windows (Version 8.2). EC50, ErC50 and the corresponding 95 % confidence intervals for each 24-hour exposure interval were calculated by non-linear regression.

The 72-hour cell density and growth rate data were evaluated for normality and homogeneity of variance (p=0.01) using the Shapiro-Wilk's and Levene's tests, respectively. All data met the assumptions for normality and homogeneity of variance; therefore, the treatment groups were compared to the negative control using Dunnett's test (p=0.05). The results of the statistical analyses, as well as an evaluation of the concentration-response pattern, were used to determine the NOAEC relative to each parameter at 72 hours.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50, ErC50 and NOAEC values are given below based on mean determined concentrations.

Table B.9.2.6.1.1-25: Toxicity of HMPA to *Pseudokirchneriella subcapitata* exposed for 72 hours to HMPA

Endpoint	HMPA [mg test item/L]
EC50 (cell density)	> 115
ErC50 (growth rate)	> 115
NOAEC (cell density)	60
NOAEC (growth rate)	60

Concentrations of HMPA in the samples were determined using a HPLC/MS. Calibration standards of HMPA, ranging in concentration from 1.00 to 10.0 mg HMPA/L, were prepared in freshwater algal medium using a stock solution of HMPA in methanol. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The method limit of quantitation (LOQ) for these analyses was defined as 1.00 mg HMPA/L. The analytical results are given below.

Table B.9.2.6.1.1-26: Measured concentrations of HMPA in freshwater algal medium samples

Nominal concentration [mg HMPA/L]	Sampling time [hours]	Measured concentration [mg HMPA/L]	Percent of nominal [%]	Mean measured concentration [mg HMPA/L]	Mean percent of nominal [%]
-	0	< LOQ	-	-	-
	72	< LOQ	-		
7.5	0	7.92	106	7.3	97
	72	6.60	88.0		
15	0	14.1	94.1	14	93
	72	13.9	92.8		
30	0	29.8	99.4	29	97
	72	27.7	92.4		
60	0	62.5	104	60	100
	72	57.5	95.8		
120	0	110	91.7	115	96
	72	120	100		

Although the measured concentrations of test item in test medium always ranged between 80 and 120 % of nominal, the ecotoxicological endpoints were evaluated using the mean measured concentrations of the test item.

B. OBSERVATIONS

At test initiation, algal cells appeared normal. After 72-hours of exposure there were no noticeable changes in cell morphology in any of the tested concentrations when compared to the control. No flocculation or aggregation of cells or adherence of cells to test chambers were observed.
The results showed that the algal growth was not inhibited at the measured test item concentrations of 7.3, 14, 29 and 60 mg HMPA/L, and was inhibited slightly at the measured test item concentration of 115 mg HMPA/L

Table B.9.2.6.1.1-27: Percentage inhibition of growth rate and cell density to *P. subcapitata* exposed for 72 hours to HMPA (mean measured)

	Control	HMPA [mg test item/L]				
	-	7.3	14	29	60	115
Mean number of algae cells (10000/ml)	298.8	319.2	294.9	286.5	273.1	186.4*
Inhibition growth rate (0-72 h) [%]	-	-1	0	1	2	8*
Inhibition cell density (0-72 h) [%]	-	-7	1	4	9	38*

* There were statistically significant differences (p<0.05) in comparison to the negative control replicates.

The mean cell density in the control flasks increased by a factor greater than 16 within three days, and the factor was 299. The coefficient of variation of average specific growth rate in the control replicates during the whole test period did not exceed 7%, and it was 0.96%. The mean percent coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) did not exceed 35%, and it was 23.4%.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	299
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	23.4%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.0%

The biomass in the control cultures increased by a factor of ≥16 (actual: 299), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 23.4%) and the coefficient of variance for the whole test period it was ≤ 7% (actual: 1.0%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC10 and EC20 was performed (Positon Paper No. CA 8.2.6.1/020).

Re-calculated EC10 and EC20 values based on nominal test concentrations

Endpoint (0 – 72 hours)	HMPA [mg/L]	
	Yield	Growth rate
EC10 (95% CI)	57.8 (40.7 – 82.1)	> 120
EC20 (95% CI)	80.4 (56.1 – 116)	> 120

CI = confidence interval

Assessment and conclusion by RMS:

The study is valid. All validity criteria were met according to OECD 201 (2011) guideline. As concentrations were maintained between ± 20% of nominal, endpoints are expressed as nominal concentrations (mean measured concentrations were chosen in the study). RMS accepts the statistical analysis of the endpoints conducted by applicant (see study summary and RMS opinion below).

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.6.1/020
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 139A-396A on the toxicity of Hydroxymethyl phosphonic acid (HMPA) to *Pseudokirchneriella subcapitata* under static conditions
Report No	110054-005
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number: 139A-396A
Author: [redacted]
Substance: HMPA (hydroxymethylphosphonic acid)
Title: HMPA (hydroxymethylphosphonic acid): A 72-Hour Toxicity Test with the Freshwater Alga (*Pseudokirchneriella subcapitata*)
Completion date: 11-Oct-2011
Test guideline(s): EU Directive 92/69/EEC, Method C.3., OECD 201 (2011)
GLP: yes
Testing facility: Wildlife International, Ltd., Easton, Maryland 21601 USA
Sponsor: Monsanto Company, St. Louis, Missouri 63167; USA

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72 h EC10 and EC20 values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 139A-396A ([redacted] 2011) was statistically evaluated for the effects of HMPA (hydroxymethylphosphonic acid) on the organism *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*). The organisms were exposed for 72 hours to the following concentrations of HMPA: 7.5, 15, 30, 60 and 120 mg HMPA/L, and the measured concentrations were 7.3, 14, 29, 60 and 115 mg HMPA/L respectively. Additionally, a control was tested in parallel.

The report states the 72-h EC50 for yield and growth rate to be > 115 mg HMPA/L based on mean measured concentrations, corresponding to > 120 mg HMPA/L based on nominal concentrations. The NOEC was determined to be 60 mg HMPA/L for growth rate and cell density.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

The data used to derive the 72-h EC10 and EC20 were obtained from the original study report.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive Effect Concentrations that have 10 and 20% effects on growth rate and yield of the test subjects (EC10 and EC20), a non-linear 3-parameter normal CDF (Cumulative Distribution Function) model for growth rate and yield and regression analysis was performed.

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Result are provided in the table below:

Validity criteria acc. to OECD 201 (2011)	**Required (0 - 72 h)**	**Obtained (0 - 72 h)**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	299
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	23.4%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 7%.	≤7%	1.0%

For yield, the parameters for the 3 parameter normal CDF model are estimated as b0: 300.355, b1: 1.762, and b2: 0.326.

For growth rate, the parameters for the 3 parameter normal CDF model are estimated as b0: 1.902, b1: 2.122, and b2: 0.438.

According to the statistical parameters; F (2, 3) = 46.773; p(F) = <0.001; R2 = 0.817 the EC10 and EC20 for yield and F (2, 3) = 65.380; p(F) = <0.001; R2 = 0.865 the EC10 and EC20 for growth rate, calculations should be considered valid.

After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.237 for yield and 0.321 for growth rate as shown in Appendix 2 of this report.

The obtained EC10 and EC20 effect of HMPA on growth rate and yield on *Pseudokirchneriella subcapitata* values are presented in the table below.

Recovery of test concentrations ranged from 94.1 to 106% for fresh solutions and from 88.0 to 100% for spent solutions. Therefore, endpoints are given based on nominal concentrations.

Table B.9.2.6.1.1-28: Re-calculated EC10 and EC20 values based on nominal test concentrations

Endpoint (0 – 72 hours)	HMPA [mg/L]	
	Yield	Growth rate
EC10 (95% CI)	57.8 (40.7 – 82.1)	> 120
EC20 (95% CI)	80.4 (56.1 – 116)	> 120

III. CONCLUSION

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid

The calculated EC10 and EC20 values are 57.8 and 80.4 mg/L, respectively for yield and > 120 and > 120 mg/L for growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. RMS agrees with ECx calculations.

72h NOErC = 60 mg HMPA/L (nom)
72h ErC10 >120 mg HMPA/L (nom)
72h ErC20 >120 mg HMPA/L (nom)
72h ErC50 >120 mg HMPA/L (nom)

72h NOEyC = 60 mg HMPA/L (nom)
72h EyC10 = 57.8 mg HMPA/L (nom)
72h EyC20 = 80.4 mg HMPA/L (nom)
72h EyC50 > 120 mg HMPA/L (nom)

B.9.2.6.2. Effects on growth on additional algal species

Data point	CA 8.2.6.2/001
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae*
Report No	AB0503/J
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline	Deviations to OECD 201 (2011): Major: - Raw data is provided as optical density, however a correlation with biomass is not provided.
Previous evaluation	Yes, accepted in RAR (2015)

GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Applicant: Supportive RMS : not reliable.

Summary

The toxicity of glyphosate acid to the blue-green alga *Anabaena flos-aquae* was determined in a 120-hour, static test. Algae were exposed to glyphosate acid at nominal concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg test item/L, A control group consisting of culture medium without test item was also prepared in parallel.

The test vessels were 250 mL conical flask containing 100 mL of test or control medium. Six vessels were prepared for the control, and three replicate vessels at each concentration of glyphosate acid. Each replicate test vessel was inoculated with a nominal cell density of 2.05×10^4 cells/mL. All vessels were incubated at 24 ± 1°C under continuous illumination for 120 hours.

After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel and algal cell densities were determined by spectrophotometrically. The pH-values in the test and control media, were determined at the beginning and at the end of the test. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values.

The 72 h EbC50 (based on nominal concentrations) for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg test item/L, the 72 h ErC50 was 22 mg/L and the 72-hour NOEbC and NOErC values were both 12 mg test item/L. The 120 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 15 mg test item/L. The 120 h ErC50 was 38 mg/L.

A satisfactory correlation between optical density and biomass cannot be made as the report does not provide a calibration curve. Therefore, this study is considered supportive by the applicant. As validity criteria and effects on biomass and growth rates could not be checked, RMS considers this study not reliable for risk assessment..

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%

Vehicle of test material/media: Cell growth medium

Test organism:

Species:	Blue-green alga *Anabaena flos-aquae*
Initial cell concentration:	2.05×10^4 cells/mL
Source:	Brixham Environmental Laboratory culture from strain CCAP 1403/13A, Culture Centre of Algae and Protozoa, Institute of Freshwater Ecology. Windermere Laboratory, Far Sawrey,

Ambleside, Cumbria, UK

Environmental conditions:

Temperature:	24.1-24.2°C (measured by thermometer)
	The hourly temperature measured automatically remained within 24 ± 1°C
Photoperiod:	Continuous illumination
Light intensity:	3600 lux
pH:	3.5 – 7.2 at the start of the test
	3.6 – 8.2 at the end of the test

B. STUDY DESIGN

Experimental dates: 4 March – 9 March 1996

Experimental treatments
The toxicity of glyphosate acid to the blue-green alga *Anabaena flos-aquae* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (0.75, 1.5, 3.0, 6.0, 12, 24, 48, 96 mg test item/L) and a control consisting of culture medium without test item.
The stock solution of nominal concentration of 96 mg test item/L was prepared by adding 192 mg of glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 0.75, 1.5, 3.0, 6.0, 12, 24, and 48 mg test item/L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel.
The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution, with six replicate vessels prepared for the control group with culture medium only and three replicate vessels prepared for each concentration of glyphosate acid. Each replicate test vessel was inoculated with 1.120 mL of the inoculum culture to give a nominal cell density of 2.05×10^4 cells/mL. The culture vessels were incubated at 24 ± 1°C under continuous illumination for 120 hours. A blank vessel (without algal inoculum) containing control medium and single blank vessels for each test concentration were also incubated concurrently.

Observations
The algal cell densities were determined by spectrophotometric adsorbance, using a Uvikon 860 UV/visible spectrophotometer. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank solution absorbance was subtracted from that of the test culture to obtain the algal absorbance reading. At the start of the test, the absorbance of a range of dilutions of the inoculum culture was used to determine the relationship between absorbance and cell density. The pH-values were measured in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
One-way analysis of variance, and Dunnett's procedure.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate acid ranged from 98 to 110 % of the nominal values. On the basis of the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

Table B.9.2.6.2.2-1: Toxicity of glyphosate acid to *Anabaena flos-aquae* (nominal values)

Endpoint	Glyphosate acid [mg a.s./L]	
	0 – 72 hours	0 -120 hours
ErC50 (95% CI)	22 (8.8 – >96)	38 (20 – >96)
EbC50 (95% CI)	8.5 (2.6 – 28)	15 (9.7 – 27)
NOErC	12	12
LOErC	24	24
NOEbC	12	12
LOEbC	24	24

B. OBSERVATIONS

Glyphosate acid inhibited cell growth of the fresh water algae *Anabaena flos-aquae* after 120 hours at test concentrations of 24, 48 and 96 mg test item/L (nominal).

Table B.9.2.6.2.2-2: Mean areas under the growth curve

Nominal concentration [mg a.s./L]	0-3 day		0-4 day		0-5 day	
	Mean area under growth curve	% of control	Mean area under growth curve	% of control	Mean area under growth curve	% of control
Control	0.4	-	1.5	-	3.5	-
0.75	0.4	91	1.5	103	3.6	105
1.5	0.3	85	1.5	99	3.6	102
3.0	0.3	80	1.4	94	3.5	99
6.0	0.3	82	1.4	94	3.5	100
12	0.3	76	1.3	87	3.3	93
24	0.0*	6	0.0*	2	0.0*	1
48	0.0*	5	0.0*	2	0.0*	1
96	0.0*	5	0.0*	2	0.0*	1

* Significant difference from the culture control (P=0.05)

Table B.9.2.6.2.2-3: Mean growth rates

Nominal concentration [mg a.s./L]	0-3 day		0-4 day		0-5 day	
	Mean growth rate	% of control	Mean growth rate	% of control	Mean growth rate	% of control
Control	1.392	-	1.331	-	1.139	-
0.75	1.365	98	1.357	102	1.145	101
1.5	1.336	96	1.355	102	1.139	100
3.0	1.328	95	1.344	101	1.141	100
6.0	1.321	95	1.342	101	1.144	100
12	1.299	93	1.321	99	1.138	100
24	0.231*	17	0.216*	16	0.251*	22
48	0.231*	17	0.173*	13	0.139*	12
96	0.231*	17	0.173*	13	0.139*	12

* Significant difference from the culture control (P=0.05)

III. CONCLUSIONS

The 72 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg test item/L, the 72 h ErC50 was 22 mg/L and the 72-hour NOEbC and NOErC values were 12 mg test item/L, respectively. The 120 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 15 mg test item/L. The 120 h

ErC50 was 38 mg/L.

Assessment and conclusion by applicant:
The nominal based 72 h EbC50 for *Anabaena flos-aquae* exposed to glyphosate acid was 8.5 mg a.s/L, the 72 h ErC50 was 22 mg a.s./L and the 72-hour NOEbC and NOErC values were 12 mg a.s./L, respectively. Raw data of the study is given in optical density. A satisfactory correlation between optical density and biomass cannot be made as the report does not provide a calibration curve.
Therefore, this study is considered supportive. Another valid study with *Anabaena flos-aquae* is available.

Assessment and conclusion by RMS:

Validity criteria, biomass and growth rates could not be checked as only optical densities measured by spectrophotometry were presented in raw data and no calibration curve of the relationship between optical density and cell density is available. Additionaly low pH were recorded.
As validity criteria and effects on biomass and growth rates could not be checked, RMS considers this study not reliable for risk assessment.

Data point	CA 8.2.6.2/002
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Anabaena flos-aquae*
Report No	1092-02-1100-4
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Minor: - Initial cell density of 3×10^3 cells/mL was below the recommended density of 10^4 cells/mL for *Anabaena flos-aquae*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of glyphosate technical on *Anabaena flos-aquae* were evaluated in a 7-day static toxicity test. The test comprised five nominal test concentrations of 10, 18, 32, 56 and 100 mg test item/L (mean measured test concentration: 9.7, 18.1, 32.6, 55.1 and 102.2 mg test item/L). In addition, a control (untreated culture medium) was tested.

The test flasks were inoculated with cells from a seven-days-old pre-culture of *Anabaena flos-aquae* with an initial test cell density of 3000 cells/mL. The test was performed in 500 mL volumetric flasks,

containing each 100 mL test solution. The test concentrations and the control were prepared in three replicates. The test flasks were placed in an incubator and maintained over several generations for 7 days. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation.

Cells were counted on test days 2, 3, 4, and 7 after test initiation by using a Coulter counter. On the basis of the mean cell count, the percentage inhibition was determined and the ECx values calculated using of the algal growth curve as determined by inverse estimation least squares linear regression.

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 79.8% for the nominal test concentration of 18 mg test item/L to 99.5% for the highest nominal test concentration of 100 mg test item/L. At the lowest nominal concentration of 10 mg test item/L, however, a slight algal growth increase of 5.4% relative to control was observed.

The 7-day EC50 for *Anabaena flos-aquae* exposed to glyphosate technical was calculated to be 4.4 mg test item/L. The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical evaluation (CA 8.2.6.2/003, [redacted] 2020) addressing the calculation of valid 72-h EC10, EC20 and EC50 as well as the NOEC values was conducted for the algae study 1092-02-1100-4 ([redacted] 1987) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.
The author concluded that calculated EC10, EC20 and EC50 values are 9.97, 11.8 and 16.4 mg/L for yield and 7.63, 12.7 and 33.4 mg a.e./L for growth rate, respectively and the NOEC was determined to be 10 mg a.e./L for yield and growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.
RMS agreed with these ECx values but considered that endpoint at 96h should have been proposed given that effects at 96h seems stronger (data gap).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6%
Water solubility	1.2 % at 25°C
Vehicle of test material/media:	Dilution water (AAP medium)

Test organism:

Species:	*Anabaena flos-aquae*
Initial cell concentration:	3000 cells/mL
Source:	In-house culture

Environmental conditions:

Temperature:	24 ± 2°C
Photoperiod:	24 h light
Light intensity	2153 ± 323 Lux
pH:	7.5 ± 01

Conductivity: Not stated
Hardness: Not stated

B. STUDY DESIGN

Experimental dates of work: 20 April to 27 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with five nominal concentrations (10, 18, 32, 56 and 100 mg test item/L) and three replicates per test item treatment group. Test concentrations were prepared by adding the required volumes of the stock solution to AAP medium. A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 500 mL volumetric flasks, containing each 100 mL test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 3000 cells/mL. Flasks were kept in an incubator at a temperature of 24 ± 2°C. Flasks were manually shaken on every working day.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Based on the mean cell count, the percentage inhibition was determined. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation. Samples of test media were taken at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations

To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50% inhibition and the associated 95% confidence intervals were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on mean measured concentrations.

Table B.9.2.6.2.2-4: Toxicity of glyphosate technical to *Anabaena flos-aquae*

Endpoint	Glyphosate technical [mg a.e./L]
EC50 (7 day)	4.4

Chemical analyses were performed on samples of the test solutions to quantify glyphosate technical in the test solution. The mean measured concentrations were 9.7, 18.1, 32.6, 55.1 and 102.2 mg test item/L, corresponding to 97.0%, 100.6%, 101.9%, 98.4% and 102.2% of the nominal test concentrations of 10, 18, 32, 56 and 100 mg test item/L respectively.

B. OBSERVATIONS

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 79.8% for the nominal test concentration of 18 mg test item/L to 99.5% for the highest nominal test

concentration of 100 mg test item/L. At the lowest nominal concentration of 10 mg test item/L, however a slight algal growth increase of 5.4% relative to control was observed.
As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Table B.9.2.6.2.2-5: Percentage of growth inhibition of *Anabaena flos-aquae* exposed to glyphosate technical for 7 days

Nominal concentrations [mg test item/L]	**Control**	**10**	**18**	**32**	**56**	**100**
Measured concentrations [mg a.e./L]	**-**	**9.7**	**18.1**	**32.6**	**55.1**	**102.2**
Mean number of algae cells on Day 7 [× 1000 cells/mL]	1486.66	1566.667	300.0	10.0	8.333	7.667
Mean inhibition (7 days) [%]	-	-5.4	79.8	99.3	99.4	99.5

Table B.9.2.6.2.2-6: Mean cell densities and percentage of inhibition of cell growth of *Selenastrum capricornutum* exposed for 72 and 96 hours to glyphosate

	Control	**Glyphosate acid [mg test item/L]**				
Test parameters	-	**10**	**18**	**32**	**56**	**100**
Mean cell densities (0-72 h) (× 10000 cells/mL)	8	8.2	3.2	1.03	0.767	0.867
Mean cell densities (0-96 h) (× 10000 cells/mL)	38.3	27.1	3.27	0.867	0.666	0.733
Mean yield inhibition (0-72 h) [%]	-	-2.6	62.3	90.5	93.9	92.6
Mean yield inhibition (0-96 h) [%]	-	4.3	89.4	98.0	98.7	98.5
Mean growth rate inhibition (0-72 h) [%]	-	-0.75	27.9	62.3	71.4	67.8
Mean growth rate inhibition (0-96 h) [%]	-	0.95	47.5	76.7	82.4	80.3

III. CONCLUSIONS

Assessment and conclusion by applicant:
Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	**Required (0 - 72 h)**	**Obtained (0 - 72 h)**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	27
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	20.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	6.4%

The biomass in the control cultures increased by a factor of ≥16 (achieved: 27), the coefficient of variance for section specific growth rates was ≤ 35% (achieved: 20.6%) and the coefficient of variance for the whole test period it was ≤ 10% (achieved: 3.4%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC10, EC20, EC50, NOEC and LOEC was performed (Positon Paper No. CA 8.2.6.2/003).

Recovery of mean measured concentrations ranged from 91 to 108%. Therefore, endpoints are based on nominal test concentrations.

Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal test concentrations

Endpoint (0 – 72 hours)	**Glyphosate technical [mg a.e./L]**	
	Yield	**Growth rate**
EC10 (95% CI)	9.97 (7.21 – 11.7)	7.63 (3.08 – 11.9)
EC20 (95% CI)	11.8 (9.35 – 13.4)	12.7 (6.71 – 17.7)
EC50 (95% CI)	16.4 (14.7 – 18.1)	33.4 (25.7 – 43.7)
NOEC	10	10
LOEC	18	18

CI = confidence interval

Assessment and conclusion by RMS:

The study is valid. All validity criteria were met according to OECD 201 (2011) guideline. As no algal measurement was done at 24h, RMS adapted the calculation of the mean coefficient of variation for section-by-section between day 0 and day 2 according to OECD 201. RMS found that the biomass in the control cultures increased by a factor of 26.7, that the mean coefficient of variation for section-by-section specific growth rate is 21.8% and that the coefficient of variation of average specific growth rates is 3.1%.

The study was conducted over 7 days, which is considered too long to derive an endpoint for risk assessment of algae in the sense of the EFSA 2013 aquatic guidance document. Moreover, given that the exponential growth decreased after 4 days in the control in the study, endpoints calculated after this time point are not considered sufficiently robust since growth of algae is not optimal.
Nevertheless, raw data allows calculating endpoints for lower durations (48h, 72h and 96h). Endpoints recalculated by applicant in the statistical re-evaluation are for 72h. RMS noted that effects seems stronger at 96h. This should be considered when setting the endpoints. The statistical analysis of the endpoints conducted by applicant at 72h is accepted by RMS (see study summary and RMS opinion below). RMS considers that endpoint at 96h should have been proposed given that effects at 96h seems stronger (data gap).

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.6.2/003
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 1092-02-1100-4 on the toxicity of Glyphosate technical to *Anabaena flos-aquae* under static conditions
Report No	110054-006
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number:	1092-02-1100-4
Author:	[redacted]
Substance:	Glyphosate
Title:	The toxicity of glyphosate technical to *Anabaena flos-aquae*
Completion date:	20-Apr-1987
Test guideline(s):	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Testing facility:	Malcolm Pirnie, Inc., White Plains, NY, USA
Sponsor:	Monsanto Agricultural Company, Chesterfield, MO, USA

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and the 72-h EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 1092-02-1100-4 ([redacted] 1987) was statistically evaluated for the effects of Glyphosate technical on the organism *Anabaena flos-aquae*. The organisms were exposed for 7 days to the following concentrations of Glyphosate technical: 10, 18, 32, 56 and 100 mg a.s. /L (nominal concentrations, units equivalent to mg a.e./L). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects (EC10 EC20 and EC50), for yield and growth rate probit analysis using linear maximum likelihood regression was used.

NOEC for yield and growth rate was estimated by Welsh-t-test After Bonferroni-Holm Correction (one-sided smaller, p = 0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The validity criteria according to the current guideline OECD 201 (2011) were met and this study is considered valid for risk assessment purposes. Results are provided in the table below:

Table B.9.2.6.2.2-7: Validity Criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	27
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	20.6%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	6.4%

Recovery of mean measured concentrations ranged from 91 to 108% of nominal. Therefore, endpoints are based on nominal test concentrations.

For yield, the parameters for the probit model are estimated as slope b: 5.95612; Intercept a: -7.22883.

For growth rate, the parameters for the probit model are estimated as slope b: 1.99737; Intercept a: -3.04435.

According to the statistical parameters; Chi2(13) = 0.59361; p(Chi²): 1.000; F(1,13) = 34.365; p(F) <0.001; r²: 0.726 for yield; and Chi2(13) = 1.26237; p(Chi²): 1.000; F(1,13) = 34.400; p(F) <0.001; r²: 0.726 for growth rate. Based on these values the EC10, EC20 and EC50 for yield and growth rate calculations should be considered valid.

The obtained EC10 EC20 and EC50 values on the effect of Glyphosate technical on growth rate and yield of *Anabaena flos-aquae* are presented in the table below.

Table B.9.2.6.2.2-8: Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal test concentrations

Endpoint (0 – 72 hours)	Glyphosate technical [mg a.e./L]	
	Yield	Growth rate
EC10 (95% CI)	9.97 (7.21 – 11.7)	7.63 (3.08 – 11.9)
EC20 (95% CI)	11.8 (9.35 – 13.4)	12.7 (6.71 – 17.7)
EC50 (95% CI)	16.4 (14.7 – 18.1)	33.4 (25.7 – 43.7)
NOEC	10	10
LOEC	18	18

CI = confidence interval

III. CONCLUSION

3. Assessment and conclusion

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated EC10, EC20 and EC50 values are 9.97, 11.8 and 16.4 mg/L for yield and 7.63, 12.7 and 33.4 mg a.e./L for growth rate, respectively. The NOEC was determined to be 10 mg a.e./L for yield and growth rate. The statistical parameters showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. RMS agrees with ECx calculations.
Endpoints at 72 hours are as follows:

72h ErC10 = 7.63 mg glyphosate acid/L (nom)
72h ErC20 = 12.7 mg glyphosate acid/L (nom)
72h ErC50 = 33.4 mg glyphosate acid /L (nom)

72h EyC10 = 9.97 mg glyphosate acid /L (nom)
72h EyC20 = 11.8 mg glyphosate acid /L (nom)
72h EyC50 = 16.4 mg glyphosate acid /L (nom)

RMS considers that endpoint at 96h should have been proposed given that effects at 96h seems stronger (data gap).

Data point	CA 8.2.6.2/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to freshwater diatom *Navicula pelliculosa*
Report No	AB0503/K
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 135.5 %, instead of ≤ 35%. Minor: - Initial cell density of 3×10^3 cells/mL, which is below the recommended density of 10^4 cells/mL for *Navicula pelliculosa.*
Previous evaluation	Yes, applicant : accepted in RAR (2015) RMS :not valid in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary

The toxicity of glyphosate acid to the freshwater diatom *Navicula pelliculosa* was determined in a 120-hours static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution.

The test was performed in 6 replicate cultures of the culture medium control and 3 replicate cultures of each concentration of glyphosate acid. The initial cell density was 0.300×10^4 cells/mL. The cell densities were determined by electronic particle counting, using a Coulter counter after 1, 2, 3, 4, and 5 days. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

The mean measured concentrations of glyphosate acid ranged from 106 to 111% of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results. Glyphosate acid inhibited cell growth of the fresh water diatom *Navicula pelliculosa* after 120 hours at test concentrations of 32, 56 and 100 mg test item/L in terms of area under growth curve and growth rates.

The 72 hours EbC50 for *Navicula pelliculosa* exposed to glyphosate acid was 16 mg test item/L; the 72 hours ErC50 was 17 mg test item/L. The 120 hours EbC50 and ErC50 were both 17 mg test item/L.

The NOErC and LOErC for *Navicula pelliculosa* after 72 hours and 120 hours of exposure were both 18 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 72 hours of exposure were 3.2 and 5.6 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 120 hours of exposure were <1.8 and 1.8 mg test item/L, respectively. The validity criteria according to current guideline OECD 201 were not met. Therefore, this study is not considered

valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%

2. Vehicle of test material/media: Vehicle: Cell growth medium

3. Test organism:

Species:	Freshwater diatom *Navicula pelliculosa*
Initial cell concentration:	3×10^3 cells/mL
Source:	Brixham Environmental Laboratory culture from strain UTEX 667

4. Environmental conditions:

Temperature:	24.0-24.1°C (measured by thermometer). The hourly temperature measured automatically remained within 24 ± 1°C
Photoperiod:	Continuous illumination
Light intensity:	4560 lux
pH:	3.7 – 8.3 at the start of the test 3.7 – 8.7 at the end of the test

B. STUDY DESIGN

Experimental dates: 29 January - 3 February 1996

Experimental treatments

The toxicity of glyphosate acid to the freshwater diatom *Navicula pelliculosa* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.8, 3.2, 5.6, 10, 18, 32, 56, and 100 mg test item/L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution.

The stock solution of nominal concentration of 100 mg test item/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg test item/L. To each test and blank vessel 100 mL of the appropriate test solution were dispensed.

The test was performed in 6 replicate cultures of the culture medium control and 3 replicate cultures of each concentration of glyphosate acid. Each replicate test vessel was inoculated with 0.915 mL of the inoculum culture to give a nominal cell density of 0.300×10^4 cells/mL. The culture vessels were incubated at 24 ± 1°C under continuous illumination for 120 hours. During incubation, the cells were kept in suspension by continuous shaking.

Observations

The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was

measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
One-way analysis of variance, and Dunnett's post-hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EbC50 and ErC50 (72 hours and 120 hours), corresponding NOEC and LOEC values are given below based on nominal concentrations.

Table B.9.2.6.2.2-9: Toxicity of glyphosate acid to *Navicula pelliculosa*

Endpoint	Glyphosate acid [mg test item/L]	
	0 – 72 hours	**0 -120 hours**
ErC50 (95% CI)	17 (13 - 24)	17 (12 - 24)
EbC50 (95% CI)	16 (12 - 22)	17 (13 - 24)
NOErC	18	18 A
LOErC	32	32
NOEbC	3.2	<1.8 B
LOEbC	5.6	1.8

A Effects observed in the 5.6 mg test item/L test concentrations were due to growth enhancement. No inhibitory effects were observed below the nominal 32 mg test item/L test concentration.
B Effects observed in the 1.8, 3.2, 5.6, and 10 mg test item/L test concentrations were due to growth enhancement. No inhibitory effects were observed below the nominal 32 mg test item/L test concentration.

Analytical data: The mean measured concentrations of glyphosate acid ranged from 106 to 111% of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

B. OBSERVATIONS

Glyphosate inhibited cell growth of the fresh water diatom *Navicula pelliculosa* after 120 hours at test concentrations of 32, 56 and 100 mg glyphosate acid/L in terms of area under growth curve and growth rates.

Table B.9.2.6.2.2-10: Mean algal densities of *Navicula pelliculosa* after treatment with glyphosate acid

Glyphosate acid [mg test item/L]	Algal cell density [× 10^4 cells ml-1]				
	Day 0	**Day 1**	**Day 3**	**Day 4**	**Day 5**
Control	0.321	0.169	18.2	93.2	170
1.8	0.321	0.109	22.0	165	197
3.2	0.321	0.271	3.43	171	156
5.6	0.321	3.38	32.0	190	166
10	0.321	0.347	29.8	177	160
18	0.321	0.136	10.9	74.2	187
32	0.321	0.060	0.071	0.181	0.237
56	0.321	0.008	0.005	0.035	0.212
100	0.321	0.001*	0.006	0.001*	0.147

*Algal density measurement for replicate was lower than the blank solution

Table B.9.2.6.2.2-11: Mean area under growth curve and mean growth rates of *Navicula pelliculosa* exposed for 72 hours and 120 hours to glyphosate acid

	Control	Glyphosate acid [mg test item/L]							
Test parameters	**-**	**1.8**	**3.2**	**5.6**	**10**	**18**	**32**	**56**	**100**
Mean areas under the growth curve (0 – 72 h)	11.0	12.1	16.7	22.6*	17.9*	5.8	-0.7*	-0.8*	-0.8*
Mean areas under the growth curve (0 – 72 h) % of control	-	111	153	206	163	53	-6	-7	-7
Mean growth rates (0 – 72 h)	1.346	1.409	1.485	1.534	1.510	1.175	-0.504*	-1.366*	-1.309*
Mean growth rates (0 – 72 h) % of control	-	105	110	114	112	87	-37	-102	-97
Mean areas under growth curve (0 – 120 h)	197.7	285.8*	278.6*	311.3*	288.9*	178.4	-1.0*	-1.3*	-1.4*
Mean areas under growth curve (0 – 120 h) [%] of control	-	145	141	157	146	90	0	-1	-1
Mean growth rates (0 – 120 h)	1.255	1.284	1.237	1.250	1.243	1.274	-0.061*	-0.083*	-0.156*
Mean growth rates (0 – 120 h) [%] of control	-	102	99	100	99	102	-5	-7	-12

* Significant difference from the control (p=0.05)

III. CONCLUSIONS

The NOErC and LOErC for *Navicula pelliculosa* after 72 hours and 120 hours of exposure were both 18 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 72 hours of exposure were 3.2 and 5.6 mg test item/L, respectively. The NOEbC and LOEbC for *Navicula pelliculosa* after 120 hours of exposure were <1.8 and 1.8 mg test item/L, respectively.

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	**Required (0 - 72 h)**	**Obtained (0 - 72 h)**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	56.6
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	135.5%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	4.4%

The biomass in the control cultures increased by a factor of ≥16 (actual: 56.6), the coefficient of variance for section specific growth rates exceeded 35% (actual: 135.5%), for the whole test period it was ≤ 10% (actual: 4.4%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to the current guideline OECD 201 were not met. Therefore, this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:
The study is not valid as the mean coefficient of variation for section-by-section specific growth rates in the control exceeded the trigger of 35%.

Data point	CA 8.2.6.2/005
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Navicula pelliculosa*
Report No	1092-02-1100-2
Document No	
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - Applicant: The biomass in the control cultures increased by a factor of 9 instead of ≥16, and the coefficient for the whole period was 10.1% instead of ≤ 10% RMS : biomass in the control cultures increased by a factor of 30.1, the mean coefficient of variation for section-by-section specific growth rate is 14.6% and the coefficient for the whole period was 5.8%Minor: - Nominal cell density of 3×10^3 cells/mL was below the recommended density of 10^4 cells/mL for *Navicula pelliculosa*,
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Applicant : Invalid RMS : valid.

Summary

The effects of glyphosate technical on *Navicula pelliculosa* were evaluated in a 7-day static toxicity test. After a range-finding test, suspensions of *Navicula pelliculosa* were exposed to five nominal concentrations encompassing 10, 18, 32, 56 and 100 mg test item/L (measured: 10.6, 19.1, 33.6, 56.1 and 103 mg glyphosate technical/L). In addition, a control with the test medium (without test substance) was tested.

The test flasks were inoculated with cells from a 7-days-old pre-culture of *Navicula pelliculosa* with an initial test cell density of 1000 cells/mL. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. The test concentrations and the control were prepared in 3 replicates. The test flasks were placed in the incubator and maintained over several generations for 7 days. The temperature

was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation.

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Three counts per replicate were made. On the basis of the mean cell count, the percentage inhibition was determined and the ECx values calculated using of the algal growth curve as determined by inverse estimation least squares linear regression.

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 97.9 to 99.7% for the nominal test concentrations of 56 mg test item/L and 100 mg test item/L respectively. At or below the nominal test concentration of 32 mg test item/L no algal growth inhibition was observed. Rather slight algal growth increases of 2.0% and 7.7% were observed for the nominal concentrations of 18 mg test item/L and 32 mg test item/L respectively.
Because the biomass in the control cultures increased by a factor of <16, and the coefficient of variation for the whole period > 10%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid by applicant for risk assessment purposes. RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline. However, only a 7d EC50 based on yield is available in the study report, and RMS considered that 72h ECx (EC10, EC20 and EC50) based on yield and growth rate should be calculated (data gap).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility	1.2% at 25°C
Vehicle of test material/media:	Dilution water

Test organism:

Species:	*Navicula pelliculosa*
Initial cell concentration:	3×10^3 cells/mL
Source:	In-house culture

Environmental conditions:

Temperature:	20 ± 2°C
Photoperiod:	24 h light, 4306 ± 650 Lux
pH:	7.5 ± 0.1
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates of work: 13 April to 20 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. Based on the preliminary test results, the main test was performed with five

nominal concentrations (10, 18, 32, 56 and 100 mg test item/L). Test concentrations were prepared by adding the required volumes of the stock solution to AAP/Si (medium with silicon) medium. A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 250 mL volumetric flasks, containing each 50 ml test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 1.11×10^6 cells/mL. Flasks were kept in an incubator at a temperature of 20 ± 2°C and were continuously shaken at 100 oscillations per minute.

Observations
Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Three counts per replicate were made. All counts were multiplied by the appropriate conversion factors (for sample dilution and volume counted) to yield cells/mL. Samples ranging in volume from 0.1 to 2.0 mL, depending upon the expected population density, were collected aseptically using an automatic micropipette with sterile tips. Based on the mean cell count, the percentage inhibition was determined and the ECx values calculated using the algal growth curve as determined by inverse estimation least squares linear regression. The temperature was measured daily and the pH was adjusted to 7.5 ± 0.1 at test initiation. Samples of test media were made at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations
To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 percent inhibition and the associated 95% confidence limits were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on mean measured concentrations.

Table B.9.2.6.2.2-12: Toxicity of glyphosate technical to *Navicula pelliculosa*

Endpoint	Glyphosate technical [mg test item/L]
EC50 (7 day)	24.9

Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 10.6, 19.1, 33.6, 56.1 and 103 mg glyphosate technical/L, corresponding to 106.0, 106.1, 105.0, 100.2 and 103.0 % of the nominal test concentrations of 10, 18, 32, 56 and 100 mg glyphosate technical/L respectively. The ecotoxicological endpoints were evaluated using measured concentrations of the test item.

B. OBSERVATIONS

Observations:
The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 97.9 to 99.7% for the nominal test concentrations of 56 mg test item/L and 100 mg test item/L respectively. At or below the nominal test concentration of 32 mg test item/L no algal growth inhibition was observed. Rather slight algal growth increases of 2.0% and 7.7% were observed for the nominal concentrations of 18 mg test item/L and 32 mg test item/L respectively.

Table B.9.2.6.2.2-13: Percentage growth inhibition of *Navicula pelliculosa* exposed to glyphosate technical for 7 days

Nominal concentrations [mg test item/L]	Measured concentrations [mg test item/L]	Mean number of algae cells (day7) [× 1000 cells/mL]	Mean inhibition (7 days) [%]
Control	Control	3020	-
10	10.6	2933	2.9
18	19.1	3080	-2.0
32	33.6	3253	-7.7
56	56.1	63.5	97.9
100	103	8	99.7

III. CONCLUSIONS

The 7-day EC50 for *Navicula pelliculosa* exposed to glyphosate technical was calculated to be 24.9 mg test item/L.

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated according to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	9
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	29.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤ 10%	10.1%

The biomass in the control cultures increased by a factor of <16 (actual: 9), the coefficient of variance for section specific growth rates was ≤ 35% (actual: 29.1%), for the whole test period it exceeded 10% actual: 10.1%). Because the biomass in the control cultures increased by a factor of <16, and the coefficient of variation for the whole period > 10%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

RMS checked the validity criteria and found that all validity criteria were met according to OECD 201 (2011) guideline. As no algal measurement was done at 24h, RMS adapted the calculation of the mean coefficient of variation for section-by-section between day 0 and day 2 according to OECD 201 guideline and obtained the following results for 72h:

Day 0	Day 2	Day 3	Coefficient of variation for section-by-section				
cells/mL	cells/mL	cells/mL	Mean	SD	CV	Mean CV	Criteria : < 35 %
3000	34000	108000	1.18	0.04	3.47	14.6	OK

3000	28000	70000	1.02	0.14	13.95		
3000	43000	93000	1.05	0.40	37.66		
3000	34000	108000	1.18	0.04	3.47		
3000	28000	70000	1.02	0.14	13.95		
3000	43000	93000	1.05	0.40	37.66		

For others validity criteria, RMS found that the biomass in the control cultures increased by a factor of 30.1 and that the coefficient of variation of average specific growth rates is 5.8%. Therefore, the study is considered valid by RMS.

As only a 7d EC50 based on yield is available in the study report, 72h ECx (EC10, EC20 and EC50) based on yield and growth rate should be calculated (data gap).

Data point	CA 8.2.6.2/006
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Report No	AB0503/I
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984) US EPA Guideline 540/09-82-020 (1982)
Deviations from current test guideline	Deviation from the guideline 201 (2011): None
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.e./L) and a control consisting of culture medium without test item. The test comprised six replicate cultures of the culture medium control and three replicate cultures of each concentration of glyphosate acid. The initial nominal cell density was 1.00×10^4 cells/mL. The culture vessels were incubated at 20 ± 1°C for 120 hours.

The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The pH-values were determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system. The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

The mean measured concentrations of glyphosate acid ranged from 94 to 106% of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

Considering the statistical re-analysis (CA 8.2.6.2/007, [redacted] 2020), the calculated 72 h- EC10, EC20 and EC50 values are 5.22, 6.38 and 8.99 mg a.s./L, respectively for yield and 1.87, 2.98, and 13.5

mg a.s./L for growth rate, respectively, based on glyphosate acid. NOEC was determined to be 5.6 mg for yield as well as for growth rate. The statistical parameters showed that these values can be considered reliable for use in the risk assessment.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6 %
Vehicle of test material/media:	Cell growth medium

Test organism:

Species:	Marine alga *Skeletonema costatum*, strain CCAP 1077/1C
Initial cell concentration:	1.00×10^4 cells/mL
Source:	Culture centre of algae and protozoa, Dunstaffnage Marine Laboratory, Oban, Argyll, UK

Environmental conditions:

Temperature:	20.0 - 20.1°C (measured by thermometer). The hourly temperature measured automatically remained within 20 ± 1°C
Photoperiod:	16 h light / 8 h dark
Light intensity:	4340 lux
pH:	7.1 – 8.1 at the start of the test
	8.1 – 8.8 at the end of the test

B. STUDY DESIGN

Experimental dates: 5 February – 10 February 1996

Experimental treatments

The toxicity of glyphosate acid to the marine alga *Skeletonema costatum* was determined in a 120-hour, static test. The test incorporated 8 nominal concentrations of glyphosate acid (1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg test item/L) and a control consisting of culture medium without test item. The test vessels were conical glass flasks of 250 mL nominal capacity containing 100 mL of test solution. The stock solution of nominal concentration of 56 mg test item/L was prepared by adding glyphosate acid directly to 2000 mL sterile culture medium. Appropriate aliquots of this stock solution were diluted to prepare the lower test concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, and 32 mg test item/L. 100 mL of the appropriate test solution were dispensed to each test and blank vessel.

The test was performed in six replicate cultures of the culture medium control and three replicate cultures of each concentration of glyphosate acid. The initial nominal cell density was 1.00×10^4 cells/mL. The culture vessels were incubated at 20 ± 1°C for 120 hours. During incubation, the cells were kept in suspension by continuous shaking.

Observations

The cell densities were determined by electronic particle counting, using a Coulter counter. After 1, 2, 3, 4, and 5 days, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density. The pH-values were

determined in the test media at the beginning and at the end of the test. The temperature in the incubator was measured daily with a thermometer, and hourly with an automatic recording system.
The concentrations of glyphosate acid in the test solutions were measured at the start and at the end of the test.

Statistical calculations
One-way analysis of variance, and Dunnett's post-hoc test.

II. RESULTS AND DISCUSSION

A. FINDINGS

The mean measured concentrations of glyphosate acid ranged from 94 to 106% of the nominal values. Based on the analytical results the nominal test concentration values were used for the calculation and reporting of all results.

The EC50 (72 h and 120 h), NOEC and LOEC values are given below based on nominal concentrations.

Table B.9.2.6.2.2-14: Toxicity of glyphosate acid to *Skeletonema costatum* (nominal values)

Endpoint	Glyphosate acid [mg test item/L]	
	0 – 72 hours	**0 -120 hours**
ErC50 (95% CI)	18 (10 – 42)	24 (12 – >56)
EbC50 (95% CI)	11 (7.1 – 20)	12 (7.6 – 19)
NOErC	1.8	10
LOErC	3.2	18
NOEbC	1.8	1.8
LOEbC	3.2	3.2

B. OBSERVATIONS

Glyphosate inhibited cell growth of the marine algae *Skeletonema costatum* after 120 hours at test concentrations of 18, 32 and 56 mg glyphosate acid/L; mean area under growth curve was affected at 10, 18, 32 and 56 mg glyphosate acid/L.

Table B.9.2.6.2.2-15: Mean cell densities and percentage of inhibition of cell growth of *Skeletonema costatum* exposed for 120 hours to glyphosate acid

	Control	Glyphosate acid [mg test item/L]							
Test parameters	**-**	**1.0**	**1.8**	**3.2**	**5.6**	**10**	**18**	**32**	**56**
Mean areas under the growth curve (0 - 72 h)	37.4	38.0	38.9	29.5*	34.2	17.9*	2.8*	2.3*	1.5*
Mean areas under the growth curve (0 - 72 h) % of control	-	102	104	79	92	48	8	6	4
Mean growth rates (0 - 72 h)	1.423	1.433	1.443	1.322*	1.387	1.111*	0.362*	0.295*	0.188*
Mean growth rates (0 - 72 h) % of control	-	101	101	93	97	78	25	21	13
Mean areas under the growth curve (0 - 96 h)	97.6	99.0	100.8	84.5	92.6	62.6	4.6	3.3	1.9
Mean areas under the growth curve (0 - 96 h) % of control	-	101	103	87	95	64	5	3	2
Mean growth rates (0 - 96 h)	1.113	1.112	1.113	1.128	1.121	1.122	0.317*	0.190*	0.087*
Mean growth rates (0 - 96 h) % of control	-	100	100	101	101	101	28	17	8
Mean areas under growth curve (0 - 120 h)	162.2	162.7	163.3	149.5*	156.9	132.1*	7.1*	4.0*	2.2*
Mean areas under growth curve (0 - 120 h) [%] of control	-	100	101	92	97	81	4	2	1
Mean growth rates (0 - 120 h)	0.882	0.879	0.869	0.873	0.875	0.905	0.315*	0.115*	0.055*
Mean growth rates (0 - 120 h) [%] of control	-	100	99	99	99	103	36	13	6

* Significant difference from the culture control (p=0.05)

III. CONCLUSIONS

The 72 h EbC50 for *Skeletonema costatum* exposed to glyphosate acid was 11 mg test item/L; the 72 h ErC50 was 18 mg/L (nominal). The 120 h EbC50 was 12 mg test item/L; the 120 h ErC50 was 24 mg test item/L. The 72-hour NOEbC and NOErC values were 1.8 mg/L, respectively. All endpoints are based on nominal test concentrations.

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

Validity criteria

Validity criteria acc. to OECD 201 (2011)	**Required (0 - 72 h)**	**Obtained (0 - 72 h)**
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	72

The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	33.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	4.3%

The biomass in the control cultures increased by a factor of ≥16 (achieved: 72), the coefficient of variance for section specific growth rates was ≤ 35% (achieved: 33.1%) and the coefficient of variance for the whole test period it was ≤ 10% (achieved: 4.3%). The validity criteria according to the current guideline OECD 201 were met and this study is considered valid for risk assessment purposes.

A statistical re-evaluation addressing EC10, EC20, EC50, NOEC and LOEC was performed (Positon Paper No. CA 8.2.6.2/007).
Recovery of test concentrations ranged from 94 to 106%. Therefore endpoints are based on nominal.

Re-calculated EC10, EC20, EC50, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	**Glyphosate acid [mg a.e./L]**	
	Yield	**Growth rate**
EC10 (95% CI)	5.22 (2.44 – 6.70)	1.87 (1.18 – 2.62)
EC20 (95% CI)	6.38 (2.90 – 7.73)	2.98 (2.86 – 5.26)
EC50 (95% CI)	9.00 (7.58 – 10.4)	13.5 (10.8 – 20.7)
NOEC	5.6	5.6
LOEC	10.0	10.0

CI = confidence interval
n.d= not determined

Assessment and conclusion by RMS:
The study is valid. All validity criteria were met according to OECD 201 (2011) guideline. The study was conducted over 5 days, which is considered too long to derive an endpoint given that the exponential growth decreased after 3 days in the control in the study.
Endpoints recalculated by applicant in the statistical re-evaluation are for 72h, which seems acceptable to RMS as the exponential growth decreased after 3 days and in addition, ECx values at 96h are expected to be in the same range as the 72h ECx values based on the growth rate inhibition data. The statistical analysis of the endpoints conducted by applicant is accepted by RMS (see study summary and RMS opinion below).
RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.6.2/007
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL5684/B on the toxicity of Glyphosate acid to *Skeletonema costatum* under static conditions
Report No	110054-007
Document No	-
Guidelines followed in study	OECD 201 (2011)
Deviations from current test guideline	Deviations to current guideline OECD 201 (2011): None
Previous evaluation	No, not previously evaluated
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Study number:	AB0503/I
Author:	[redacted]
Substance:	Glyphosate acid
Title:	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum*
Completion date:	10-Feb-1996
Test guideline(s):	OECD Guideline No. 201 (1984); US EPA Guideline 540/09-82-020 (1982) Re-evaluated according OECD 201 (2011)
GLP:	Yes, conducted under GLP/Officially recognised testing facilities
Testing facility:	Brixham Environmental Laboratory, Brixham Devon, UK
Sponsor:	ZENECA Agrochemicals, Surrey, UK

B. STUDY DESIGN

Dates of work: April 2020

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and 72-h EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study BL5684/B ([redacted] 1996) was statistically evaluated for the effects of Glyphosate acid on the marine alga *Skeletonema costatum.* The organisms were exposed for 120-hours to the following concentrations of glyphosate acid: 1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from the original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations
Models providing best fit to the respective data were selected and are as follows:
In order to derive the 72-h Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects, a 3-parameter logistic CDF (Cumulative Distribution Function) model was used for yield and for growth rate and a non-linear regression analysis was performed.

NOEC levels were determined by Welsh-t-test After Bonferroni-Holm Correction for yield, and Williams Multiple Sequential t-test for growth rate (one-sided smaller; p= 0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC_{10} and EC_{20} and EC_{50} values were calculated to fulfil the data requirements according to regulation EU 283/2013.Validity parameters are provided in the table below:

Table B.9.2.6.2.2-16: Validity Criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	72
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤35%	33.1%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤10%	4.3%

For yield, the parameters for the 3 parameter logistic CDF model are estimated as: b0: 53.393, b1: 8.991; b2: 4.038.
For growth rate, the parameters for the logit analysis are estimated as slope b: 2.46938; intercept a: -2.86713.

According to the statistical parameters; $F(2, 6) = 147.118$; $p(F) < 0.001$; $R^2 = 0.910$ for yield.
After non-linear regression no lack of fit was detected for the function ($p(F|\text{Lack of Fit}) = 0.069$
For growth rate, statistical parameters for goodness of fit are: $Chi^2(22) = 0.54119$; $p(Chi^2)$: 1.000; $F(1,22) = 97.922$, $p(F) < 0.001$; $R^2 = 0.817$.

Therefore, the obtained EC_{10}, EC_{20} and EC_{50} calculations are considered valid. The values are presented in the table below.

Considering yield, there is a statistically significant difference to control at test concentrations 3.2, 10.0, 18, 23, and 56 mg/L. No statistically significant effect is determined for the intermediate test concentration of 5.6 mg/L. As this does not follow a dose response scenario and continuous effects are observed from 10.0 mg/L and all higher concentration levels, the NOEC is set to 5.6 mg/L for yield.

For growth rate, % inhibition at 72 hours was -0.7, -1.5, 6.9, 2.4, 21.7, 74.8, 79.2, and 86.8% compared to the control for test concentrations 1.0, 1.8, 3.2, 5.6, 10, 18, 32, and 56 mg a.s./L, respectively.

However, statistically significant effects have been determined for all test concentrations, except for the two lowest levels. Even inhibition of 6.9 and 2.4% at 3.2 and 5.6 mg/L are statistically determined to show an effect. Based on the fact that the inhibition values at these test item concentrations were below 10% these significances were considered to be not scientifically relevant according to [redacted] (1998).

Recovery of mean measured test concentrations ranged from 94 to 106% of nominal. Therefore, endpoints are based on nominal.

Table B.9.2.6.2.2-17: Re-calculated EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values based on nominal concentrations

Endpoint (0 – 72 hours)	Glyphosate acid [mg a.s./L]	
	Yield	Growth rate
EC_{10} (95% CI)	5.22 (2.44 – 6.70)	1.87 (1.18 – 2.62)
EC_{20} (95% CI)	6.38 (3.90 – 7.73)	2.98 (2.86 – 5.26)
EC_{50} (95% CI)	8.99 (7.58 – 10.4)	13.5 (10.8 – 20.7)
NOEC	5.6	5.6
LOEC	10.0	10.0

CI = confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:
The validity criteria according to the current guideline OECD 201 were met and this study is considered valid.

The calculated 72 h- EC_{10}, EC_{20} and EC_{50} values are 5.22, 6.38 and 8.99 mg a.s./L, respectively for yield and 1.87, 2.98, and 13.5 mg a.s./L for growth rate, respectively, based on glyphosate acid. NOEC was determined to be 5.6 mg a.s./L for yield as well as for growth rate. The statistical parameters showed that these values can be considered reliable for use in the risk assessment.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. RMS agrees with ECx calculations.
Endpoints set for the study CA 8.2.6.2/006 ([redacted] 1996) :

72h NOErC = 5.6 mg glyphosate acid/L (nom)
72h ErC10 = 1.87 mg glyphosate acid/L (nom)
72h ErC20 = 2.98 mg glyphosate acid/L (nom)
72h ErC50 = 13.5 mg glyphosate acid /L (nom)

72h NOEyC = 5.6 mg glyphosate acid/L (nom)
72h EyC10 = 5.22 mg glyphosate acid /L (nom)
72h EyC20 = 6.38 mg glyphosate acid /L (nom)
72h EyC50 = 8.99 mg glyphosate acid /L (nom)

Data point	CA 8.2.6.2/008
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Skeletonema costatum*
Report No	1092-02-1100-3
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - The biomass in the control cultures increased by a factor of 3.6 instead of ≥16, and the mean coefficient of variation for section-by-section specific growth rates in the control cultures was 78.4% instead of ≤35%
Previous evaluation	Applicant :Yes, accepted in RAR (2015) RMS: Yes, not valid in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary

The effects of glyphosate technical on *Skeletonema costatum* were evaluated in a 7-day static toxicity test. After a range-finding test, suspensions of *Skeletonema costatum* were exposed to six nominal concentrations encompassing 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg test item/L (measured: 0.24, 0.28, 0.48, 1.79 and 3.42 mg glyphosate technical/L). In addition, a control with the test medium (without test substance) was tested. The test flasks were inoculated with cells from a 7-days-old pre-culture of *Skeletonema costatum* with an initial test cell density of 104 cells/mL. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. The test concentrations and the control were prepared in 3 replicates. The test flasks were placed in the incubator and maintained over several generations for 7 days. The temperature was measured daily and the salinity was adjusted to 30‰ at test initiation. Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. On the basis of the mean cell count, the percentage inhibition was determined and the ECx values calculated using of the algal growth curve as determined by inverse estimation least squares linear regression. The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.2 % for the lowest mean measured test concentration of 0.24 mg test item/L to 95.7% for the highest test concentration of 3.42 mg test item/L. At the mean measured concentration of 0.28 mg test item/L, a sporadic growth increase of 13.6% relative to control was observed.

The 7-day EC50 for *Skeletonema costatum* exposed to glyphosate technical was calculated to be 0.64 mg test item/L.

Because the factor of exponential increase in biomass in the control cultures was <16 and the coefficient of variation for the section specific growth rate was >35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6 %
Water solubility	1.2% at 25°C
Vehicle of test material/media:	filter-sterilized distilled deionized water

Test organism:

Species:	*Skeletonema costatum*
Initial cell concentration:	10^4 cells/mL
Source:	In-house culture

Environmental conditions:

Temperature:	20 ± 2°C
Photoperiod:	14 h light / 10 h dark
Light intensity:	4306 ± 650 Lux
Salinity:	30 ‰
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates: 20 April 1987 to 27 April 1987

Experimental treatments

Prior to the main test, a range-finding test was performed with six concentrations ranging between 0.001 and 100 mg test item/L. On the basis of the preliminary test results, the main test was performed with six nominal concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg test item/L) and three replicates per test item treatment group. Test concentrations were prepared by adding the required volumes of the stock solution to synthetic seawater (prepared by adding approximately 30 grams of a commercial salt mix to 1 L of distilled deionised water). A control with the test medium (without test substance) was tested under the same conditions as in the test groups. The test was performed in 250 mL volumetric flasks, containing each 50 mL test solution. Test algae were taken from a 7-day old stock culture and were aseptically added to the test medium to obtain a nominal initial concentration of 104 cells/mL. Flasks were kept in an incubator at a temperature of 20 ± 2°C. Flasks were manually shaken each working day.

Observations

Cell counts were made using a Coulter counter on test days 2, 3, 4, and 7 after test initiation. Based on the mean cell count, the percentage inhibition was determined and the ECx values calculated using the algal growth curve as determined by inverse estimation least squares linear regression. The temperature was measured daily and the salinity was adjusted to 30‰ at test initiation. Samples of test media were made at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analysed for active substance using HPLC.

Statistical calculations

To determine the ECx values, the log of test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50% inhibition and the associated 95% confidence limits were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 value is given below based on mean measured concentrations.

Table B.9.2.6.2.2-18: Toxicity of glyphosate technical to *Skeletonema costatum*

Endpoint	Glyphosate technical [mg test item/L]
EC50 (7 day) (95% confidence limits)	0.64 (0.21 – 1.70)

Analytical data:
Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 0.24, 0.28, 0.48, 0.94, 1.79 and 3.42 mg glyphosate/L, corresponding to 240.0%, 140.0%, 120.0%, 117.5%, 111.9% and 106.9% of the nominal test concentrations of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg glyphosate/L, respectively. Therefore, ecotoxicological endpoints were evaluated using measured concentrations of the test item.

B. OBSERVATIONS

The effects of the test item on algal growth inhibition on day 7, relative to the control, ranged from 9.2% for the lowest mean measured test concentration of 0.24 mg test item/L to 95.7% for the highest test concentration of 3.42 mg test item/L. At the mean measured concentration of 0.28 mg test item/L, a sporadic growth increase of 13.6% relative to control was observed.

Table B.9.2.6.2.2-19: Percentage growth inhibition of *Skeletonema costatum* exposed to glyphosate technical for 7 days

Nominal concentrations [mg test item/L]	Measured concentrations [mg test item/L]	Mean number of algae cells (day 7) [× 1000 cells/mL]	Mean inhibition (7 days) [%]
Control	Control	360.667	-
0.1	0.24	327.333	9.2
0.2	0.28	410.667	-13.6
0.4	0.48	250.667	30.5
0.8	0.94	76.333	78.8
1.6	1.79	24.000	93.3
3.2	3.42	15.667	95.7

III. CONCLUSIONS

The 7-day EC50 for *Skeletonema costatum* exposed to glyphosate technical was calculated to be 0.64 mg test item/L, based on mean measured concentrations.

Assessment and conclusion by applicant:

The validity criteria for the study were re- evaluated to the current guideline OECD 201 (2011).

Validity criteria

Validity criteria acc. to OECD 201 (2011)	Required (0 - 72 h)	Obtained (0 - 72 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥16	3.6
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	78.4%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	≤ 10%	2.5%

The biomass in the control cultures increased by a factor of <16 (actual: 3.6), the coefficient of variance for section specific growth rates exceeded 35% (actual: 78.4%), for the whole test period it was ≤10% (actual: 2.5%). Because the factor of exponential increase in biomass in the control cultures was <16 and the coefficient of variation for the section specific growth rate was >35%, the validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

RMS checked the validity criteria and found that validity criteria were not met according to OECD 201 (2011) guideline. As no algal measurement was done at 24h, RMS adapted the calculation of the mean coefficient of variation for section-by-section between day 0 and day 2 according to OECD 201.

At 72h, the biomass in the control cultures increased by a factor of 3.6, the mean coefficient of variation for section-by-section specific growth rate is 77.8% and the coefficient of variation of average specific growth rates is 2.2%.

Therefore, the study is not considered valid by RMS.

Data point	CA 8.2.6.2/009
Report author	[redacted]
Report year	1978
Report title	Toxicity of seven test materials to the marine alga, *Skeletonema costatum*
Report No	BP-78-4-031
Document No	-
Guidelines followed in study	Environmental Protection Agency: Bioassay procedures for the ocean disposal permit program (1976)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011) Major: - Report does not provide sufficient information
Previous evaluation	Applicant: Yes, accepted in RAR (2015) RMS : study not found in previous RAR
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed
Acceptability/Reliability (RMS)	Applicant: Invalid RMS : not reliable.

Summary

The effects of seven test items, two solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45) and five liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A) on *Skeletonema costatum*, were evaluated in a 96-hour static toxicity test. The test was performed using nominal concentration encompassing 0.6, 1.0, 1.8, 3.2 and 5.6 mg test item/L for Glyphosate, BN-78-44 and 3.2, 10, 32, 100, 320 and 560 mg test item/L for Glyphosate intermediate, BN-78-45). For the liquid test item (Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.) the nominal concentration used were 0.6, 1.0, 3.2, 10, 32 and 56% effluent. Duplicate cultures were employed for each of the test concentrations and control, except in the test with Comp. 5A, in which all test concentrations and the control were triplicate. The test solutions were prepared using deionised water. The initial cell concentration was 2 x 10^4 cells/mL. Cell cultures were incubated for 96 hours at 20 ± 1°C.

Measurements of *in vivo* chlorophyll α in cultures were performed and cell counts were made at 24, 48, 72 and 96 hours after the test initiation. Due to the nature of two of the test materials, Comp. #2, BN-78-47 and Comp. #3, BN-78-48, *in vivo* chlorophyll α could not be accurately measured. Cell counts were the only growth measurement for both test items. The EC50 values were calculated in terms of chlorophyll α measurements and cell counts.

For the test item Glyphosate, BN-78-44, all test concentrations led to a reduction in both chlorophyll α content and the cell number, varying from 12% to 98%. For the test item Glyphosate intermediate BN-78-45, a reduction in chlorophyll α content and the cell number were observed from the nominal concentrations of 320 mg test item/L (for chlorophyll α) and 10 mg test item/L (for cell number), respectively. At the highest test concentrations, the reductions in both chlorophyll α content and the cell number varied from 95% to 98% for both solid test items.

For the liquid test items Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A, reductions in chlorophyll α content and/or cell number were observed from the lowest test concentration (0.6% effluent), except for Comp. 5A, for which the reduction in chlorophyll α content was observed only at or above the concentration of 10% effluent. At the highest test concentration (56% effluent), reductions in both chlorophyll α content and the cell number varied from 88% to 100% for all liquid test items.

Validity of the study could not be checked due to lack of information given in the report. The study is therefore not used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item (Description):	Glyphosate, BN-78-44 (white, crystalline solid) Glyphosate intermediate, BN-78-45 (fine, white powder) Comp. #1, BN-78-46 (clear liquid) Comp. #2, BN-78-47 (clear liquid) Comp. #3A, BN-78-48 (murky liquid) Comp. #4, BN-78-49 (clear liquid) Comp. 5A. (clear liquid)
Vehicle and/or positive control:	Dodecyl sodium sulphate (DSS)

Test organism:

Species:	*Skeletonema costatum*
Initial cell concentration	2 x 10^4 cells/mL
Source:	Environmental Protection Agency's Protection Agency's'Environmental Research Laboratory, Narragansett, Rhode Island, USA

Environmental conditions:

Temperature:	20 ± 1°C
Photoperiod:	Not stated
Light intensity:	2000 Lux
pH:	Glyphosate, BN-78-44, (8.2 – 8.5) Glyphosate intermediate, BN-78-45 (6.1 – 8.4) Comp. #1, BN-78-46 (7.6 – 8.4) Comp. #2, BN-78-47 (7.1 – 8.4) Comp. #3A, BN-78-48 (8.1 – 8.5 Comp. #4, BN-78-49 (8.0 – 8.9) Comp. 5A (8.2 – 8.5)
Dissolved oxygen:	Not stated
Conductivity:	Not stated
Hardness:	Not stated

B. STUDY DESIGN

Experimental dates: Not stated

Experimental treatments

Toxicity tests for the seven test materials were performed using nominal concentration encompassing 0.6, 1.0, 1.8, 3.2 and 5.6 mg test item/L for Glyphosate, BN-78-44, and 3.2, 10, 32, 100, 320 and 560 mg test item/L for Glyphosate intermediate, BN-78-45).For the liquid test item (Comp. #1, BN-78-46; Comp. #2, BN-78-47; Comp. #3A, BN-78-48; Comp. #4, BN-78-49 and Comp. 5A.) the nominal concentration used were 0.6, 1.0, 3.2, 10, 32 and 56% effluent. Duplicate cultures were employed for each of the test concentrations and control, except in the test with Comp. 5A, in which all test concentrations and the control were triplicate. For solid test materials, appropriate amounts were added to deionised water; the pH was adjusted to 8.0, and the materials were finally added test containers to

obtain appropriate concentrations. For liquid materials, the effluents were directly added into the test containers. To the prepared tests concentrations, the algal suspension was added to obtain an initial cell concentration of 2×10^4 cells/mL. Cell cultures were incubated for 96 hours at 20 ± 1°C.

Observations
Measurements of *in vivo* chlorophyll α in cultures were performed by using a fluorometer and cell counts were made by a means of a haemocytometer and a standard microscope at 24, 48, 72 and 96 hours after the test initiation. Due to the nature of two of the test materials, Comp. #2, BN-78-47, and Comp. #3, BN-78-48, *in vivo* chlorophyll α could not be accurately measured. Cell counts were the only growth measurement for both test items. The EC50 values were calculated in terms of chlorophyll α measurements and cell counts. A separate test was conducted, in which cultures of the alga were exposed to the reference toxicant dodecyl sodium sulphate under the same test conditions stated above.

Statistical calculations
The EC50 values were calculated by linear regression in a Probit data analysis. The salinity growth data were analysed by Student's t-test at α = 0.05.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC50 values are given below based on nominal concentrations.

Table B.9.2.6.2.2-20: Toxicity to *Skeletonema costatum*

Test materials		**EC50 (96 h) (95% confidence interval) [% effluent or mg test item/L]**
Glyphosate, BN-78-44 (CL)	chlorophyll α	1.2 (0.6 – 2.3)
	cell counts	1.3 (0.7 – 2.5)
Glyphosate intermediate BN-78-45 (CL)	chlorophyll α	>100 <320
	cell counts	140 (51 - 379)
Comp. #1, BN-78-46	chlorophyll α	13 (6.1 - 27)
	cell counts	15 (6.8 - 33)-
Comp. #2, BN-78-47	chlorophyll α	n.d.
	cell counts	> 1<10
Comp. #3A, BN-78-48	chlorophyll α	n.d.
	cell counts	> 3.2 < 10-
Comp. #4, BN-78-49	chlorophyll α	12 (6.8 - 23)
	cell counts	19 (7.8 - 48)
Comp. 5A.	chlorophyll α	14 (7.6 - 25)
	cell counts	4.5 (2.2 - 9.1)

n.d.= not determined

B. OBSERVATIONS

For the test item Glyphosate, BN-78-44, all test concentrations led to a reduction in both chlorophyll α content and the cell number, varying from 12% to 98%. For the test item Glyphosate intermediate, BN-78-45, a reduction in chlorophyll α content and the cell number were observed from the nominal concentrations of 320 mg test item/L (for chlorophyll α) and 10 mg test item/L (for cell number). At the highest test concentrations, the reductions in both chlorophyll α content and the cell number varied from

95% to 98% for both solid test items. For the liquid test items Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A, reductions in chlorophyll α content and/or cell number were observed from the lowest test concentration (0.6 % effluent), except for Comp. 5A, for which the reduction in chlorophyll α content was observed only at or above the concentration of 10% effluent. At the highest test concentration (56% effluent), reductions in both chlorophyll α content and the cell number varied from 88 % to 100 % for all liquid test items.

Table B.9.2.6.2.2-21: Lethal effects of Glyphosate, BN-78-44, on *Skeletonema costatum*

Glyphosate, BN-78-44 [mg test item/L]	Control	0.6	1.0	1.8	3.2	5.6
Chlorophyll α (96 h) [%]	-	-12	-42	-84	-93	-98
Cell number [%] (96 h) [%]	-	-12	-35	-69	-90	-97

Table B.9.2.6.2.2-22: Lethal effects of Glyphosate intermediate, BN-78-45, on *Skeletonema costatum*

Glyphosate intermediate, BN-78-45 [mg test item/L]	Control	3.2	10	32	100	320	560
Chlorophyll α (96 h) [%]	-	+10	+7	+19	+10	-90	-95
Cell number [%] (96 h) [%]	-	+44	-3	-7	-14	-89	-90

Table B.9.2.6.2.2-23: Lethal effects of Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49, and Comp. 5A on *Skeletonema costatum*

Test items [% effluent]	Control	0.6	1.0	3.2	10	32	56	Sal. b
Comp. #1, BN-78-46								
Chlorophyll α (96 h) [%]	-	+2	-5	+10	-33	-95	-100	-83 a
Cell number [%] (96 h) [%]	-	+5	-21	+25	-18	-97	-99	-85 a
Comp. #2, BN-78-47								
Chlorophyll α (96 h) [%]	-	-	-	-	-	-	-	-
Cell number [%] (96 h) [%]	-	+7	-1	-50	-79	-80	-88 a	-
Comp. #3A, BN-78-48								
Chlorophyll α (96 h) [%]	-	-	-	-	-	-	-	-
Cell number [%] (96 h) [%]	-	-20	-21	+11	-94	-99	-98	-48 a
Comp. #4, BN-78-49								
Chlorophyll α (96 h) [%]	-	-10	+14	-10	-5	-74	-l00	-
Cell number [%] (96 h) [%]	-	-24	+24	-16	-1	-68	-97	-
Comp. 5A								
Chlorophyll α (96 h) [%]	-	+8	+3	+5	-24	-97	-100	-
Cell number [%] (96 h) [%]	-	-10	-15	-3	-56	-96	-100	-

a significantly different (α=0.05) from the control
b Salinity control

Table B.9.2.6.2.2-24: Lethal effects of the toxic reference Dodecyl sodium sulfate on *Skeletonema costatum*

Dodecyl sodium sulfate [mg test item/L]	Control	1	2	3
Chlorophyll α (96 h) [%]	-	0	-57	-81
Cell number [%] (96 h) [%]	-	-4	-55	-79

III. CONCLUSION

The effects of seven glyphosate-related test items on *Skeletonema costatum* were studied in an acute toxicity test. For the solid test items (Glyphosate, BN-78-44, and Glyphosate intermediate, BN-78-45), the EC50 values varied from 1.2 mg test item/L to 320 mg test item/L. For the liquid test items (Comp. #1, BN-78-46, Comp. #2, BN-78-47, Comp. #3A, BN-78-48, Comp. #4, BN-78-49 and Comp. 5A.), the EC50 values varied between 1 % effluent to 19 % effluent.

Assessment and conclusion by applicant:
Validity of the study could not be checked due to lack of information given in the report. The study is not used for risk assessment.

Assessment and conclusion by RMS:
Validity criteria, biomass and growth rates could not be checked as only percentage changes in cell number and chlorophyll a compared to control were presented in the study report. In addition, the study is not GLP, analytical measurements of tested concentrations were not conducted and the exact composition of the 7 tested items is not clear, as no analytical certificate are available. Therefore, RMS considers this study not reliable for risk assessment.

Data point	CA 8.2.6.2/010
Report author	[redacted]
Report year	1996
Report title	Alga, Growth Inhibition Test to *Nitzschia palea*
Report No	960606FH
Document No	-
Guidelines followed in study	OECD Guideline No. 201 (1984)
Deviations from current test guideline	Deviations from the guideline OECD 201 (2011): Major: - the coefficient of variance for section specific growth rates exceeded 35% (actual: 72.7%).
Previous evaluation	Applicant: Yes, accepted in RAR (2015) RMS : Yes, not valid in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Summary
The effects of glyphosate on *Nitzschia palea* were evaluated in a 96-hour static toxicity test, at seven nominal concentrations of 0.32, 1.0, 3.2, 10, 32, 100 and 320 mg test item/L and a control. Three replicate vessels were prepared per concentration level and control. The flask containing 10 mL of test or control medium were inoculated with algal cells to obtain an initial cell density of 1.0 - 1.4 × 10^4 cells/mL. The temperature was measured continuously, and the pH was determined at the beginning and end of the test.

At test start (0 h) and after 24, 48, 72 and 96 hours cell density was determined by chlorophyll-fluorescence and growth inhibition was calculated. EC10 and EC50 value for biomass (EbC) and growth rate (ErC) inhibition were calculated using Probit analysis whereas the EC0 (NOEC) values were

deducted from the dose-response-relationship.

The 96 h ErC50 for *Nitzschia palea* exposed to glyphosate was calculated to be 11.90 mg test item/L. The 96 h EbC50 was 4.47 mg test item/L. The NOEC (biomass & growth rate) were both determined to be 1.0 mg test item/L.

The validity criteria according to the current guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	White/crystalline
Lot/Batch #:	01/06/96
Purity:	96.7%
Density:	Not specified
Water solubility:	12g/L at 20°C
Vehicle of test material/media:	Cell growth medium

Test organism:

Species:	*Nitzschia palea* (Kützing)
Initial cell concentration:	1.0 – 1.4 × 10^4 cells/mL
Source:	Pflanzenphysiologisches Institut der Universität Göttingen, Göttingen, Germany

Environmental conditions:

Temperature:	21.5 – 23.8°C
Photoperiod:	24 h light
Light intensity:	70 – 90 µE/m2 s
Light quality:	Fluorescent tube, radium NL 58w/31, Spectralux Warmton
pH:	7.78 – 8.72 (control replicates) 7.71 – 8.58 at 0.32, 1.0, 3.2 and 10 mg/L 6.43 – 7.74 at 32 mg/L 5.81 – 6.74 at 100 mg/L 3.20 – 3.22 at 320 mg/L
Conductivity:	not stated
Hardness:	not stated

B. STUDY DESIGN

Experimental dates: 14 October – 18 October 1996

Experimental treatments
Prior to the main test, a range-finding test was performed using concentrations of 0.01, 0.1, 1.0, and 10

mg test item/L. The test flasks were inoculated with cells from a three-day-old pre-culture of *Nitzschia palea* to obtain an initial cell density of 1.0 -1.4 × 10^4 cells/mL.
On the basis of the preliminary test results, the main test was performed with seven test item treatment rates, 0.32, 1.0, 3.2, 10, 32, 100 and 320 mg test item/L. A control with the test medium (without test substance) was tested under the same conditions. The test was performed in 20 mL plastic cuvettes containing 10 mL test medium in static conditions. The test concentrations and the control were prepared in three replicates. The test cultures were mixed every 2 h for 15 min at 70 rpm with shaker. The temperature was measured continuously, and the pH was determined at the beginning and end of the toxicity test.

Observations
At test start (0 h) and after 24, 48, 72 and 96 hours growth of cell density was determined by chlorophyll-fluorescence and algal growth inhibition was calculated.

At test start and test termination, samples of test media were taken for analysis of the active ingredient from 0.32, 1.0, 3.2, 10, 32, 100 and 320 mg test item/L treatments. All samples were analysed for active substance using a validated HPLC.

Table B.9.2.6.2.2-25: Measured pH values were as follows:

Nominal conc. [mg/L]	Start	end
Control	7.78	8.72
0.32	7.84	8.58
1.0	7.81	8.58
3.2	7.77	8.24
10	7.71	7.97
32	6.43	7.74
100	5.81	6.74
320	3.22	3.20
320 – pH adjusted	7.97	8.09

Statistical calculations
The EC10 and EC50 value for biomass (EbC) and growth rate (ErC) inhibition were calculated using Probit analysis whereas NOEC values were deducted from the dose-response-relationship.

II. RESULTS AND DISCUSSION

A. FINDINGS

The ErC50, EbC50 and NOEC values are given below based on nominal concentrations.

Table B.9.2.6.2.2-26: Toxicity of glyphosate to *Nitzschia palea*

Endpoint	Glyphosate technical [mg test item/L]
0 - 96 h ErC50	11.90
0 – 96 h ErC10	3.11
0 - 96 h EbC50	4.47
0 - 96 h EbC10	2.12
NOEC (growth rate)	1.0
NOEC (biomass)	1.0

The analytical recovery rates at the beginning of the test were in the range of 78% and 108% of the active substance. At the end of the test, recovery rates were in the range of 68% and 98%. Low recoveries

of 68 % and 71% respectively were found in the lowest test concentration and 76% to 77% recoveries were found at the test end for 10 mg test item/L. As the overall recovery rates were >80%, the report presents data based on nominal concentrations.

B. OBSERVATIONS

The results of the main test showed that the algal growth was completely inhibited at a nominal test item concentration of 320 mg test item/L No inhibition effects were observed at and below a concentration of 1 mg test item/L. The effects on growth rate and biomass are below.

Table B.9.2.6.2.2-27: Percentage inhibition of growth rate and biomass of *Nitzschia palea* exposed for 96 hours to glyphosate

Glyphosate technical [mg test item/L]	C pH	C	0.32	1.0	3.2	10	32	100	320
Biomass integral	4.86	213.76	219.03	215.39	90.75	17.88	-6.26	-11.76	-36.67
Inh. biomass (0-96 h) [%]	97.73	-	-2.47	-0.76	57.55	91.63	100	100	100
Growth rate (0-96 h)	0.14	0.75	0.74	0.76	0.56	0.29	0.09	0.04	0.00
Rate related inhibition (0-96 h) [%]	81.89	-	1.07	-1.03	25.43	60.72	88.09	94.42	100

C = control; C pH = control pH (320 mg glyphosate/L pH adjusted); Inh. = inhibition

III. CONCLUSION

The 96 h ErC50 for *Nitzschia palea* exposed to glyphosate was calculated to be 11.90 mg test item/L. The 96 h EbC50 for *Nitzschia palea* was 4.47 mg test item/L. The NOEC (biomass) and NOEC (growth rate) were both determined to be 1.0 mg test item/L.

Assessment and conclusion by applicant:

Validity of the study was re-evaluated according to the current test guideline OECD 201 (2011) and EC10, EC20, and EC50, NOEC and LOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013. Due to the slow growth of test species, the study was extended to 96 hours, according to the guideline OECD 201. Therefore the validity criteria are applied to the time point 96 hours.

Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required (0 - 96 h)	Obtained (0 - 96 h)
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	16	19.9
The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%.	≤ 35%	72.7%
The coefficient of variation of average specific growth rates during the whole test period in replicate solvent control cultures must not exceed 10%.	< 10%	2.0%

The study was considered valid when compared to the guideline used at the time of study conduct.

However, compared with the current control validity criteria, the biomass in the control cultures increased by a factor of > 16 (actual: 19.9), the coefficient of variance for section specific growth rates exceeded 35% (actual: 72.7%) and for the whole test period it was ≤ 10% (actual: 2%). Because the coefficient of variation for the section specific growth rate was > 35%, the validity criteria according to guideline OECD 201 were not met and this study is not considered valid for risk assessment purposes.

Assessment and conclusion by RMS:
RMS checked the validity criteria and found that all validity criteria were not met according to OECD 201 (2011) guideline. At 72h, validity criteria are not met neither.Therefore, the study is not considered valid.

B.9.2.7. Effects on aquatic macrophytes

Data point:	CA 8.2.7/001
Report author	[redacted]
Report year	2002
Report title	IPA Salt of Glyphosate: Effects on *Lemna minor*
Report No	CEMR-1873
Document No	-
Guidelines followed in study	OECD Guideline 221
Deviations from current test guideline	Deviation from guideline OECD 221 (2006): none.
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effect of isopropylamine (IPA) salt of glyphosate on the growth of the duckweed *Lemna minor* was evaluated in a 7 day semi-static toxicity test at nominal concentrations of IPA salt of glyphosate of 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg/L, equivalent to 2.16, 4.32, 8.64, 18.0, 36.0 and 72.0 mg glyphosate acid/L. Furthermore, a negative control group with *Lemna minor* exposed to test medium without test substance (negative control) was prepared in parallel.

The test vessels were 250mL glass beakers containing 150mL of the test or control medium. The vessels were continuously illuminated. The medium in each of the test vessels was renewed twice; day 2 and 4. Growth in each vessel was determined by counting the numbers of plants and fronds on three occasions during the definitive test and measuring the dry weights of the fronds after seven days. Some visible effects (chlorosis and dark frond) were noted for all concentrations ≥ 11.7 mg/L. Analytical samples for analysis of glyphosate were collected from the three highest samples at the start and end of the test and following each media renewal (fresh and old media). Glyphosate isopropylamine salt was not detected in the control group. The mean measured content of the IPA salt ranged between 96 and 104% of nominal, the results are therefore based on nominal concentrations. Based on nominal concentrations of IPA salt of glyphosate, growth of *L. minor* was significantly inhibited at 24.3mg/L, but not affected at 11.7 mg/L.

All validity criteria according to the OECD guideline 221 were fulfilled.

The author concluded that the lowest 7-day EC_{50} for *Lemna minor* exposed to glyphosate IPA salt was calculated to be 25.5 mg/L, equivalent to 18.9 mg glyphosate acid/L. The 7-day NOEC for *Lemna minor* exposed to glyphosate IPA salt was determined to be 11.7 mg/L, equivalent to 8.64 mg glyphosate acid/L. The lowest observed effect concentration (LOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 24.3 mg/L, equivalent to 18.0 mg glyphosate acid/L.

According to the statistical reanalysis, the applicant concluded that the 7 day ErC_{50} was 30.3 mg a.e./L based on frond numbers at 7 days. The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* over a 7-day exposure period was 8.65 mg a.e./L.

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

This study is considered valid for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate isopropylamine (IPA) salt
Description:	White powder
Lot/Batch #:	1002B
Purity:	97.1% as IPA salt

2. Test organism:

Species:	*Lemna minor*
Source:	Pond in Marlow, Buckinghamshire, UK

3. Environmental conditions:

Temperature:	20.5 – 22.8°C
Photoperiod:	24 h fluorescence light
Light intensity	6600 - 8100 Lux
pH:	6.06 – 6.96
4. Dates of experimental work	Sept 30th to Nov 28th 2002

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed with six concentration levels, 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg glyphosate IPA salt/L, equivalent to 2.16, 4.32, 8.65, 18.0, 36.0 and 72.0 mg glyphosate/L. Furthermore, a negative control group with *Lemna minor* exposed to culture medium (SIS) only was run in parallel. The medium in each of the test vessels was renewed on day 2 and 4. Three replicates were prepared with 9-10 fronds (in 3-4 colonies) were used for each test concentration and control. Temperatures and pH values were measured in the test media were measured at the start of tests and at the end. In addition, temperature was monitored continuously. Analytical samples for analysis of glyphosate were collected at the start of the tests and at the end and following each media renewal. Samples were analysed using HPLC with fluorescence detection.

2. Observations: The numbers of fronds and colonies were counted on days 0 (start), 2, 4 and 7 during the definitive test. Dry weights of the fronds were determined at the end of the tests. The fronds from each vessel were collected, rinsed with de-ionised water and dried at 60°C to a constant weight. The dry weights of fronds from each vessel were measured to ±0.1 mg.

3. Statistical calculations: EC_{50} values were calculated using the LC_{50} program of Stephan *et al.*, 1986. The no-observed-effect concentration (NOEC) and the lowest- observed-effect concentration (LOEC) were based on statistical analysis of *L. minor* final frond numbers, growth rate and area under growth curve values, as well as the final biomass/dry weight, for the definitive test. Data were first tested for compliance with the assumptions of ANOVA in terms of normality of distribution and homogeneity. The treatment means were tested for significant difference from the control mean at α=0.05 using the Dunnett's test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Chemical analyses were performed on samples of the test media to quantify glyphosate in the test solution. The mean measured content of the test item always ranged between 80 and 120% of nominal.

Table B.9.2.7-1: Analytical results

Nominal concentration of IPA salt [mg/L]	0	2.92	5.93	11.7	24.3	48.6	97.2
Nominal concentration of glyphosate [mg/L]	0	2.16	4.32	8.65	18.0	36.0	72.0
Day 0 concentration (fresh)	< 0.26	2.14	4.23	8.44	17.9	36.5	74.1
Day 3 concentration (old)	-	2.08	4.18	8.26	17.0	31.5	69.5
Day 3 concentration (fresh)	< 0.26	2.31	4.33	8.81	17.7	36.6	85.2
Day 7 concentration (old)	< 0.26	1.85	3.94	8.32	17.4	34.6	70.8
Mean measured [mg/L]	<0.26	2.10	4.17	8.46	17.5	34.7	74.9
% of nominal	-	97	96	98	97	96	104

Table B.9.2.7-2: *L. minor* colony and frond numbers

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate number	Numbers of Lemna colonies				Numbers of Lemna fronds			
		Day 0	Day 2	Day 4	Day 7	Day 0	Day 2	Day 4	Day 7
0	1	3	3	4	23	9	17	35	128
	2	3	3	8	36	9	22	47	177
	3	3	3	5	24	9	18	36	138
2.92	1	3	3	4	19	9	17	29	114
	2	3	3	5	23	10	17	37	137
	3	3	3	6	24	9	20	44	154
5.83	1	3	4	8	28	9	17	39	147
	2	3	3	6	37	9	20	45	157
	3	3	3	7	26	9	22	43	148
11.7	1	3	3	6	27	9	20	36	112
	2	4	4	9	47	10	28	51	172
	3	3	4	7	29	10	23	38	114
24.3	1	3	3	7	18	10	16	28	55
	2	3	3	8	23	9	22	32	78
	3	3	3	7	18	10	20	31	61
48.6	1	3	3	4	9	9	14	19	24
	2	3	4	6	11	9	22	26	34
	3	3	3	5	8	9	15	18	24
97.2	1	3	3	5	8	10	15	17	20
	2	3	3	5	6	9	16	18	19
	3	3	3	4	4	9	12	15	17

Table B.9.2.7-3: *L. minor* growth rates

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate Number	Growth rate (0-7 days)	Frond doubling time (days)	Average growth rate (0-7 days)	Percent inhibition in growth rate relative to controls
0	1	0.379	1.83	0.398	
	2	0.426	1.63		
	3	0.390	1.78		
2.92	1	0.363	1.91	0.381	4%
	2	0.374	1.85		
	3	0.406	1.71		
5.83	1	0.399	1.74	0.402	-1%
	2	0.408	1.70		
	3	0.400	1.73		
11.7	1	0.360	1.92	0.371	7%
	2	0.406	1.71		
	3	0.348	1.99		
24.3	1	0.244	2.85	0.270	32%
	2	0.308	2.25		
	3	0.258	2.68		
48.6	1	0.140	4.95	0.157	61%
	2	0.190	3.65		
	3	0.140	4.95		
97.2	1	0.099	7.00	0.099	75%
	2	0.107	6.49		
	3	0.091	7.63		

Table B.9.2.7-4: *L. minor* area under growth curves

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate Number	Area under growth curves (0-7 days)	Average a.u.g.c. (0-7 days)	Percent inhibition in area under growth curve relative to controls
0	1 2 3	27.65 30.87 28.19	28.90	-
2.92	1 2 3	26.36 27.50 29.84	27.90	3
5.83	1 2 3	28.61 30.01 29.89	29.50	-2
11.7	1 2 3	27.88 31.28 27.99	29.05	-1
24.3	1 2 3	23.19 26.49 24.67	24.78	14
48.6	1 2 3	18.99 22.96 18.93	20.29	30
97.2	1 2 3	17.46 18.42 16.31	17.40	40

Table B.9.2.7-5: *L. minor* change in biomass/dry weight

Nominal concentration of IPA salt of glyphosate (mg/L)	Replicate Number	Total increase in dry weights of fronds over 7 days (g)	Average increase in biomass (0-7 days) (g)	Percent inhibition in biomass increase relative to controls
0	1 2 3	0.0342 0.0387 0.0343	0.0357	-
2.92	1 2 3	0.0264 0.0302 0.0362	0.0309	13%
5.83	1 2 3	0.0331 0.0334 0.0361	0.0342	4%
11.7	1 2 3	0.0343 0.0393 0.0298	0.0345	3%
24.3	1 2 3	0.0178 0.0233 0.0218	0.0210	41%
48.6	1 2 3	0.0147 0.0167 0.0122	0.0145	59%
97.2	1 2 3	0.0125 0.0122 0.0121	0.0123	66%

Visible effects were noted and described below for each concentration:

- 97.2 mg/L: some chlorosis and elongation of fronds, some fronds became very dark, algal growth apparent in all vessels on surface.
- 48.6 mg/L and 24.3 mg/L: some cholrosis and elongation of fronds, some very dark fronds.
- 11.7 mg/L: slight chlorosis and slight elongation of fronds.

- 5.83 mg/L and 2.92 mg/L: no visible effect in comparison with controls.

B. OBSERVATIONS

The results of the definitive test showed no effect on frond growth at 11.7 mg IPA salt/L and partial and statistically significant inhibition at 24.3 mg IPA salt/L. At 48.6 and 97.2 mg IPA salt/L the inhibition of frond growth was greater at 81% and 87% inhibition for final frond numbers. The validity criteria according to guideline OECD 221 are fulfilled.

The endpoints given below are based on nominal concentrations of IPA salt of glyphosate and glyphosate acid.

Table B.9.2.7-6: Toxicity of glyphosate IPA salt and glyphosate acid to *Lemna minor*

Endpoint	Glyphosate IPA-salt [mg/L]	Glyphosate acid [mg/L]
$EyC_{50,\ frond\ number}$ (7 day)	25.5 (C.I.: 11.1 – 73.4)	18.9 (C.I.: 8.2 – 54.4)
$NOECy_{frond\ number}$ (7 day)	11.7	8.65
$EyC_{50,\ dry\ weight}$ (7 day)	46.2 (C.I.: 18.6 – 1673)	34.2 (C.I.: 13.8 – 1239)
$NOECy_{\ dry\ weight}$ (7 day)	11.7	8.65
$EbC_{50,\ frond\ number}$ (7 day)	Not calculable	Not calculable
$NOECb_{\ frond\ number}$ (7 day)	11.7	8.65
$ErC_{50,\ frond\ number}$ (7 day)	42.6 (C.I.: 26.3 – 87.8)	31.6 (C.I.: 19.5 – 65.0)
$NOECr_{\ frond\ number}$ (7 day)	11.7	8.65

C.I.: confidence interval

The lowest observed effect concentration (LOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 24.3 mg IPA salt/L, equivalent to 18.0 mg glyphosate acid/L. The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7-day exposure period was 11.7 mg/L, equivalent to 8.65 mg glyphosate acid/L. The lowest 7 day EC_{50} was 25.5 mg/L with 95% confidence limits of 11.1 to 73.4 mg/L measured from final frond numbers at 7 days, equivalent to 18.9 mg glyphosate acid/L (8.22 – 54.37 mg a.s./L).

III. CONCLUSIONS

Assessment and conclusion by applicant:

The lowest 7 day EyC_{50} was 25.5 mg/L with 95% confidence limits of 11.1 to 73.4 mg/L measured from final frond numbers at 7 days, equivalent to 18.9 mg glyphosate acid/L (8.22 – 54.37 mg a.s./L).

The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* measured over a 7 day exposure period was 11.7 mg/L, equivalent to 8.65 mg glyphosate acid/L.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints.
The percent recovery nominal test concentrations are presented below.

Analytical verification of test item

Parameter	Nominal concentration of glyphosate acid equivalent [mg/L]					
	2.16	4.32	8.65	18.0	36.0	72.0

	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)					
Day 0	2.14 (99)	4.23 (98)	8.44 (98)	17.0 (94)	36.5 (101)	74.1 (103)
Day 2 aged	2.08 (96)	4.18 (97)	8.26 (95)	17.7 (98)	31.5 (88)	69.5 (97)
Day 2 fresh	2.31 (107)	4.33 (100)	8.81 (102)	17.4 (97)	36.6 (102)	85.2 (118)
Day 7 fresh	1.85 (86)	3.94 (91)	8.32 (96)	17.5 (97)	34.6 (96)	70.8 (98)
Geometric mean	2.088	4.167	8.455	17.398	34.737	74.657
	Equivalence in IPA salt nominal concentration [mg/L]*					
	2.92	5.83	11.67	24.29	48.58	97.17

* conversion factor from IPA salt to acid equivalent has been stated as 0.741 by RMS.

Analytical recovery of the test item ranged from 86 to 118% throughout the study. Therefore, calculated endpoints will be based on nominal concentrations.

Details of statistical re-evaluation are given in the position paper CA 8.2.7/002.

The 7 day ECx values for yield and growth rate based on frond numbers has been calculated based on the nominal concentrations and are provided the table below:

7-d ECx values for Yield, Growth Rate

7-day endpoints	Nominal concentration of glyphosate acid equivalent [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	18.0	7.80 (3.21 – 10.7)	10.3 (5.77 – 13.2)	16.5 (13.1 – 19.9)
Growth rate (Frond number)	8.65	8.16 (5.38 – 12.4)	12.8 (8.65 – 18.9)	30.3 (18.7 – 48.6)
Yield (Biomass dry weight)	8.65	5.72 (0.09 – 12.54)	10.3 (0.71 – 19.1)	32.1 (16.6 – 94.3)

According to the statistical reanalysis, the 7 day ErC_{50} was 30.3 mg a.e./L based on frond numbers at 7 days.
The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna minor* over a 7-day exposure period was 8.65 mg a.e./L.

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The study is valid. All validity criteria were met according to OECD 221 (2006).
The statistical analysis of the endpoints conducted by applicant is accepted by RMS (see study summary and RMS opinion below). ECx values calculated in the statistical analysis are more conservative than those calculated in the study report and are therefore kept by RMS.
Nevertheless, ErCx values based on dry weight are lacking in both study report and statistical analysis. RMS would have tried to calculate these values but it is not possible as raw data only presents the average increase in biomass for each tested concentration and not the biomass values at the beginning and at the end of the test. Nevertheless, considering that EyC50 value based on frond number is lower than the ErC50 and that the EyC50 value based on dry weight is higher than the EyC50 for frond number, ErC50 based on dry weight is not expected to be lower than the ErC50 based on frond number, suggesting that dry weight is not the more sensitive parameter. Thus it is not expected that ErCx values based on dry weight will have influence the outcome of

the endpoint to be considered for risk assessment. However this should be confirmed by submission of calculation of ErCx values based on dry weight (data gap).

Given that 60.3% reduction was measured on yield for frond number at 18 mg glyphosate acid/L, RMS disagrees with the NOEC determined for this parameter in the study and considers 8.65 mg a.e./L more appropriate.

Phytotoxic effects were not reported in details in raw data, just global descriptions as presented in the study summary. Therefore, neither an estimation of an EC50 nor a comparison with the calculated endpoints can be determined. However the NOEC of 8.65 mg a.e./L is considered appropriate to cover the phytotoxity effects (only slight chlorosis and slight elongation of fronds observed at this concentration).

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.7/002
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study CEMS-1873 on the toxicity of Glyphosate isopropylamine (IPA) salt to Lemna minor under static conditions
Report No	110054-008
Document No	-
Guidelines followed in study	OECD Guideline 221
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, not conducted under GLP (GLP is not compulsory for statistical evaluation)
Acceptability/Reliability (RMS)	Valid

Summary

A statistical evaluation addressing the calculation of valid 7 day NOEC, EC_{10}, EC_{20} and EC_{50} values was conducted for the study CEMS-1873 ([redacted] 2002) to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).
Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met (doubling time: 1.7 days, mean growth rate: 0.398/d) this study is considered valid for risk assessment purposes.
Based on the nominal concentration of glyphosate the 7-day endpoints EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 7.80, 10.3, and 16.5 mg a.e./L for yield (frond number), respectively; 8.16, 12.8, and 30.3 mg a.e./L for growth rate (frond number), and 5.72, 10.3, and 32.1 mg a.e./L for change in biomass(yield dry weight). The NOEC was determined to be 8.65 mg a.e./L.
A data gap is set by RMS for determination of ErCx values based on dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number:	CEMR-1873
Author:	[redacted]
Substance:	Glyphosate isopropylamine (IPA) salt
Title:	IPA Salt of Glyphosate: Effects on *Lemna minor*
Completion date:	05-Dec-2002
Test guideline(s):	OECD Guideline 221 (Draft version, 2002), re-evaluated according to OECD 221 (2006)
GLP:	Yes
Testing facility:	CEM Analytical Services Limited (CEMAS), Berkshire, UK
Sponsor:	Sinon Corporation, Taichung, Taiwan, R.O.C.

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7 days EC_{10}, EC_{20}, and EC_{50} values were calculated to fulfil the data requirements according to regulation EU 283/2013.
The study CEMS-1873 ([redacted] 2002) was statistically evaluated for the effects of Glyphosate isopropylamine (IPA) salt on the organism *Lemna minor*. The organisms were exposed for 7 days to the following concentrations of Glyphosate isopropylamine (IPA) salt: 2.92, 5.83, 11.7, 24.3, 48.6 and 97.2 mg glyphosate IPA salt/L, equivalent to 2.16, 4.32, 8.65, 18.0, 36.0 and 72.0 mg glyphosate acid/L. Additionally, a control was tested in parallel. The frond count data as well as change in biomass (dry weight) data for the individual control and treatment group replicates will be used to calculate the ECx values.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effects on yield and growth rate for frond number, a 3-parameter logistic CDF (Cumulative Distribution Function) model and a 3 parameter normal CDF model was used, respectively.
To estimate the effects on yield for change in biomass, probit analysis with linear maximum likelihood regression was used.

For yield and growth rate, the NOEC was determined by Multiple Sequentially-rejective Welsh-t-test after Bonferroni-Holm Correction (one sided smaller, $p > 0.01$).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

The percent recovery nominal test concentrations between 4 and 7 days are presented below.

Table B.9.2.7-7: Analytical verification of test item

Parameter	Nominal concentration of glyphosate acid equivalent [mg a.e./L]					
	2.16	4.32	8.65	18.0	36.0	72.0
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)					
Day 0	2.14 (99)	4.23 (98)	8.44 (98)	17.0 (94)	36.5 (101)	74.1 (103)
Day 2 aged	2.08 (96)	4.18 (97)	8.26 (95)	17.7 (98)	31.5 (88)	69.5 (97)
Day 2 fresh	2.31 (107)	4.33 (100)	8.81 (102)	17.4 (97)	36.6 (102)	85.2 (118)
Day 7 fresh	1.85 (86)	3.94 (91)	8.32 (96)	17.5 (97)	34.6 (96)	70.8 (98)
Geometric mean	2.088	4.167	8.455	17.398	34.737	74.657

Analytical recovery of the test item ranged from 86 to 118% of nominal throughout the study. Therefore, calculated endpoints will be based on nominal concentrations.

The mean measured content of the test item always ranged between 80 and 120% of nominal. Therefore, the endpoints given below are based on nominal concentrations of glyphosate IPA salt, expressed as glyphosate acid equivalent.

Considering frond numbers:
For yield, the parameters for the 3 parameter logistic CDF model are estimated as b0: 136.975, b1: 16.476, and b2: 2.937.
According to the statistical parameters; F (2, 4) = 101.777; p(F) = <0.001; R^2 = 0.997 the EC_{10} and $EC_{20,}$ and EC_{50} calculations should be considered valid.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.632.
For growth rate, parameters for the 3 parameter normal CDF model are estimated as b0: 0.400, b1: 0.911, and b2: 0.445.
According to the statistical parameters; F (2, 4) = 101.205.117; p(F) = <0.001; R^2 = 0.0.989 the EC_{10} and EC_{20}, calculations should be considered valid.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.177.
Considering change in biomass (dry weight):
The parameters for the logit model are estimated as slope b: 1.71104; Intercept a: -2.57759.
Statistical parameters for goodness fit are: Chi^2(15) = 0.27989; p(Chi^2): 1.000; F(1,15) = 14.751, p(F) <0.001; R^2 = 0.787 the EC_{10} , EC_{20} and EC_{50}, calculations should therefore be considered valid.

The obtained 7-day EC_{10}, EC_{20} and EC_{50} values for the effect of Glyphosate isopropylamine (IPA) salt on yield and growth rate, considering frond numbers for *Lemna minor* is presented in the table below.

Table B.9.2.7-7: 7-day ECx values for Yield, Growth Rate

7-day endpoints	Nominal concentration of glyphosate acid equivalent [mg a.e./L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	18.0	7.80 (3.21 – 10.7)	10.3 (5.77 – 13.2)	16.5 (13.1 – 19.9)
Growth rate (Frond number)	8.65	8.16 (5.38 – 12.4)	12.8 (8.65 – 18.9)	30.3 (18.7 – 48.6)
Yield (Change in biomass dry weight)	8.65	5.72 (0.09 – 12.54)	10.3 (0.71 – 19.1)	32.1 (16.6 – 94.3)

CI = confidence interval

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid.

III. CONCLUSION

Assessment and conclusion by applicant:
Based on the nominal concentration of glyphosate the 7-day endpoints EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 7.80, 10.3, and 16.5 mg a.e./L for yield (frond number), respectively; 8.16, 12.8, and 30.3 mg a.e./L for growth rate (frond number), and 5.72, 10.3, and 32.1 mg a.e./L for change in biomass. The NOEC was determined to be 8.65 mg a.e./L.

The statistical parameters demonstrate that these values can be considered reliable/valid and therefore considered for risk assessment purposes.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. RMS agrees with ECx calculations.

Endpoints to be considered are reported thereafter:

Frond number

7d NOErC = 8.65 mg glyphosate acid /L (nom)
7d ErC10 = 8.16 mg glyphosate acid/L (nom)
7d ErC20 = 12.8 mg glyphosate acid/L (nom)
7d ErC50 = 30.3 mg glyphosate acid /L (nom)

7d NOEyC = 8.65 mg glyphosate acid /L (nom)
7d EyC10 = 7.8 mg glyphosate acid /L (nom)
7d EyC20 = 10.3 mg glyphosate acid /L (nom)
7d EyC50 = 16.5 mg glyphosate acid /L (nom)

Dry weight
7d NOEyC = 8.65 mg glyphosate acid /L (nom)
7d EyC10 = 5.72 mg glyphosate acid /L (nom)
7d EyC20 = 10.3 mg glyphosate acid /L (nom)
7d EyC50 = 32.1 mg glyphosate acid /L (nom)

As previously indicated, it is not possible to propose an estimation of ECx values for phytotoxicity effects nor determine if phytotoxic effects are covered by the endpoints set for other parameters. However, RMS considered that the NOEC of 8.65 mg a.e./L is appropriate to cover the phytotoxity effects (only slight chlorosis and slight elongation of fronds observed at this concentration).

As stated previsously, a data gap is set for determination of ErCx values based on dry weight.

Data point:	CA 8.2.7/003
Report author	[redacted]
Report year	1999
Report title	Glyphosate 62% IPA-Salt, aquatic plant toxicity test using *Lemna gibba*
Report No	980909FH
Document No	-
Guidelines followed in study	Guideline ASTM E 1415- 91 (June 1991)
Deviations from current test guideline	Deviations from guideline OECD 221 (2006): Minor: - The study was performed for 14 days instead of 7.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Applicant: Valid RMS : Not reliable

Summary
The effects of glyphosate isopropylamine salt on growth of *Lemna gibba* were evaluated in a 14 day semi-static toxicity test. Based on the results of a range finding test, the definitive test was performed with five concentration levels of glyphosate IPA-salt, 6.25, 12.5, 25, 50 and 100 mg test item /L and a control with 3 replicates per test item treatment using three plants per replicate (four fronds each). Renewal of the test media was performed on day 2, 4, 7, 9 and 11. A reference substance (zinc chloride) was equally tested at 1.0, 3.2 and 10 mg/L. The number of fronds affected was determined on day 0, 7 and 14. Observation of change in colour, break-up of plants and destruction of roots was conducted on day 7 and 14. Dry biomass weight was determined on day 14 (end of the test).

Analysis of the test concentration was carried out on day 4 and day 11(freshly prepared media) and on day 7 and 14 (3 day old test media). All test concentrations and control replicates were analysed. Result showed an increase of growth of *Lemna gibba* at nominal concentrations of 6.25, 12.5 and 25 mg test item/L. Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above concentrations of 50 mg IPA salt /L.

The EC_{50} values for inhibition of front number and dry weight after 14 days were 53.56 mg IPA salt/L (equivalent to 33.42 mg glyphosate/L) and 62.59 mg IPA salt /L (equivalent to 39.06 mg glyphosate/L) respectively. The NOEC was determined to be 25 mg IPA salt /L equivalent to 15.60 mg glyphosate/L.

Analytical recovery of the test item ranged from 78 to 113% from 4 to 7 days. Therefore, calculated endpoints will be based on geometric mean concentrations.
According to the statistical reanalysis, the 7 day ErC_{50} is 34.8 mg a.e./L with 95% confidence limits of 29.7 to 41.3 mg a.e./L for frond number parameter at 7 days.
The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna gibba* over a 7-day exposure period was 14.7 mg a.e./L.
The author concluded that validity criteria according to the current guideline OECD 221 were met and that this study is considered valid for risk assessment purposes.
RMS considered the study as valid (validity criteria met) but not reliable for risk assessment (see commenting box).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate 62% IPA-Salt
name	Glyphosate Isopropylamine Salt
Description:	Clear, liquid, yellowish
Lot/Batch #:	22-9754
Purity:	62.4% glyphosate acid
Density:	1.2355 g/mL

2. positive control: Zinc chloride

3. Test organism:

Species:	*Lemna gibba*
Source:	Bundesanstalt für Gewässerkunde, Koblenz, Germany

4. Environmental conditions:

Temperature:	25 ± 2°C
Photoperiod:	24 h florescence light
Light intensity	around 4200 – 6700 lux
pH:	7.5 ± 0.1
Conductivity:	not stated
Hardness:	not stated

5. Experimental dates of work: Sept 30th 1989 to Feb 3rd 1999

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Based on the results of a range finding test, the definitive test was performed with five concentration levels, 6.25, 12.5, 25, 50 and 100 mg test item/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Three plants per replicate were used. The plants were placed in 500 mL test vessels, which already contained the 300 mL 20X-AAP test media prepared according to the guideline. The pH of the test medium was adjusted prior to the test. Three uniformly healthy-looking plants with 4 fronds each were used in each test vessel. The test was conducted under semi-static conditions with renewal of test media on day 2, 4, 7, 9 and 11. The reference substance (zinc chloride) was equally tested at 1.0, 3.2 and 10 mg/L.

2. Observations:

Biological data: The amount of the plants and fronds affected were determined on day 0, 7 and 14. Every frond that visibly projected beyond the edge of a parent frond was counted as separate frond. Observation of change in colour, break-up of plants and destruction of roots were made on day 7 and 14. Dry biomass weight was determined on day 14.

Physical data: The pH values were measured on day 0, 2, 4, 7, 9, 11 and 14. The room temperature in the test chamber was measured and recorded continuously. Sampling and analysis of the test concentration were carried out on day 4 and day 11(freshly prepared media) and on day 7 and 14 (3 day old test media). All test concentrations and control replicates were analysed.

3. Statistical calculations: EC_{50} and EC_{90} values of frond number inhibition after day 7 and 14 were calculated by Probit analysis. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dray weight, respectively, at $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 14d EC_{50} and NOEC values are given below based on nominal concentrations.

Table B.9.2.7-8: Toxicity of glyphosate isopropylamine salt to *Lemna gibba*

Endpoint	IPA salt [mg/L]		Glyphosate [mg/L]	
	Frond number	**Biomass dry weigh**	**Frond number**	**Biomass dry weight**
7d				
EC_{50}	56.26	-	-	-
95% confidence limit	45.53 - 69.53	-	-	-
NOEC	25	-	-	-
14d				
EC_{50}	53.56	62.59	33.42	39.06
95% confidence limit	42.91 - 66.85	47.94 - 81.73	26.78 - 41.71	29.91.- 51.00
NOEC	25	25	15.60	15.60

Analytical data: In freshly prepared test media the recoveries of the glyphosate varied between 78% and 86% for day 4 and 94% to 113% for day 11. In the aged test media (3 days old), 106% to 113% of the glyphosate were recovered for day 7 and 87% to 104% for day 14. As the mean measured content of the glyphosate always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the IPA salt.
RMS comment : RMS noted that no analytical verification has been made at day 0 (new), day 4(old), day 7 (new) and day 11 (old).

Table B.9.2.7-9: Analytical results

Test substance nominal [mg IPA salt/L]	Glyphosate nominal [mg/L]	**Day 4 (new media)**		**Day 7 (old media)**		**Day 11 (new media**		**Day 14 (old media)**	
		Measured conc. [mg/L]	% of nominal	Measured conc. [mg/L]	% of nominal	Measured conc. [mg/L]	% of nominal	Measured conc. [mg/L]	% of nominal
Control	-	< LOD		< LOD		< LOD		< LOD	
100	62.4	48.67	78	67.08	108	62.25	100	54.34	87
		53.12	85	66.29	106	58.88	94	57.09	91
50	31.2	26.87	86	33.44	107	31.34	100	29.32	94
		26.33	84	33.91	108	31.8	102	29.17	93
25	15.6	12.76	82	17.00	108	15.90	102	15.10	97
		12.54	80	17.12	110	15.32	98	14.64	94
12.5	7.8	6.72	86	8.29	106	8.26	106	8.01	103
		6.57	84	8.49	109	8.20	105	7.75	99
6.25	3.9	3.37	86	4.20	108	4.39	113	3.93	101
		3.21	82	4.42	113	4.10	105	4.06	104

Limit of detection of glyphosate: new media = 0.90 mg/L, old media = 0.81 mg/L.

B. OBSERVATIONS

Observations: Increase of growth was found at nominal concentrations of 6.25, 12.5 and 25 mg IPA salt/L. Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a concentration of 50 mg test item/L. Front number inhibition values after day 14 as well as biomass dry weight inhibition are presented below.

Table B.9.2.7-10: Frond numbers and inhibition values (day 0/7)

			Control	**Test item [mg/L]**				
Test item (IPA salt)				**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate				**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Frond number	Mean	Day 0	12.0	12.0	12.0	12.0	12.0	12.0
		Day 7	91.0	127.7	113.7	120.3	56.3	12.7
Increase of frond number	Mean	Day 7	79.0	115.7	101.7	108.3	44.3	0.7
Inhibition of frond number	Mean±SD	[%]	-	-46 ± 17.6	-29 ± 11.4	-37 ± 6.4	44 ± 18.1	99 ± 1.5

Table B.9.2.7-11: Frond numbers and inhibition values (day 0/14)

			Control	**Test item [mg/L]**				
Test item (IPA salt)				**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate				**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Frond number	Mean	Day 0	12.0	12.0	12.0	12.0	12.0	12.0
		Day 14	535.0	776.7	757.3	875.7	119.3	20.7
Increase of frond number	Mean	Day 14	523.0	764.7	745.3	863.7	107.3	8.7
Inhibition	Mean±SD	[%]	-	-46 ± 14.0	-43± 12.7	-65 ± 15.4	79 ± 7.5	98 ± 1.1

Table B.9.2.7-12: Dry weight after 14 days and inhibition values

			Control	Test item [mg/L]				
Test item (IPA salt)				**6.25**	**12.5**	**25**	**50**	**100**
Glyphosate				**3.90**	**7.80**	**15.60**	**31.20**	**62.40**
Biomass dry weight [mg]	Mean	Day 14	48.9	65.2	66.0	69.8	18.7	6.6
Inhibition	Mean±SD	[%]	-	-33± 10.8	-35± 9.8	-43± 12.8	62± 15.4	86± 2.7

Table B.9.2.7-13: Further observations on days 7 and 14.

Observations were made compared to the habitus of control plants

Test subst. [mg/L]	Observations on day	
	Day 7	Day 14
100	Fronds and roots smaller, fronds partially without pigmentation	Fronds and roots smaller, fronds partially without pigmentation
50	Fronds and roots smaller, fronds partially without pigmentation	Fronds and roots smaller, fronds partially without pigmentation
25	Roots smaller, fronds partially with more pigmentation	Fronds partially without pigmentation
12.5	Roots smaller, fronds partially with more pigmentation	Fronds partially without pigmentation
6.25	Roots smaller, fronds partially with more pigmentation	n.f.

n.f. = no findings

The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 are therefore fulfilled. The EC_{50} values for inhibition of front number and dry weight after 14 days were 53.56 mg IPA salt/L (equivalent to 33.42 mg glyphosate/L) and 62.59 mg IPA salt/L (equivalent to 39.06 mg glyphosate/L) respectively. The NOEC was determined to be 25 mg IPA salt/L, equivalent to 15.60 mg glyphosate/L.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Glyphosate isopropylamine salt was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 50 mg IPA salt/L. The EC_{50} values for inhibition of front number and dry weight after 14 days were 53.56 mg IPA salt/L (equivalent to 33.42 mg glyphosate/L) and 62.59 mg IPA salt/L (equivalent to 39.06 mg glyphosate/L) respectively.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints.

The percent recovery nominal test concentrations between 4 and 7 days are presented below.

Table B.9.2.7-14: Analytical verification of test item between 4 and 7 days

Parameter	Nominal concentration of glyphosate acid equivalent [mg/L]				
	3.9	7.8	15.6	31.2	62.4
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)				
Day 4 new	3.37 (86)	6.72 (86)	12.76 (82)	26.87 (86)	48.67 (78)
	3.21 (82)	6.57 (84)	12.54 (80)	26.33 (84)	53.12 (85)
Day 7 aged	4.2 (108)	8.29 (106)	17 (109)	33.44 (107)	67.08 (108)
	4.42 (113)	8.49 (109)	17.12 (110)	33.91 (109)	66.29 (106)
4 - 7 days Geometric mean	**3.8**	**7.5**	**14.7**	**29.9**	**58.2**

Analytical recovery of the test item ranged from 78 to 113% from 4 to 7 days. Therefore, calculated endpoints will be based on geometric mean concentrations.

Details of statistical re-evaluation are given in the position paper CA 8.2.7/004.

The 7-day endpoints for yield and growth rate based on frond numbers have been calculated based on the geometric mean concentrations and are provided in the table below:

Table B.9.2.7-15: 7-d endpoints for Yield frond number, Growth Rate frond number based on geometric mean measured concentrations

7-day endpoints	glyphosate [mg a.e./L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	14.7	6.42 (3.38 – 9.45)	11.1 (7.16 – 15.9)	28.1 (19.3 – 52.0)
Growth rate (Frond number)	14.7	12.8 (9.59 – 15.8)	19.1 (15.4 – 22.6)	34.8 (29.7 – 41.3)

According to the statistical reanalysis, the 7 day ErC_{50} is 34.8 mg a.e./L with 95% confidence limits of 29.7 to 41.3 mg a.e./L for frond number parameter at 7 days.

The overall no-observed effect concentration (NOEC) of the IPA salt of glyphosate to *Lemna gibba* over a 7-day exposure period was 14.7 mg a.e./L.

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The doubling time of frond number in the control is slightly above 2.5 days at 14 days (2.56 d) and below 2.5 days for 7 days (2.39 d). Nevertheless, the study is still considered valid by RMS as it is very close to the limit and the validity criterion was fulfilled at 7 days.

Effects on biomass dry weight could not be calculated for 7 days as they were only measured at 14 days.

Analytical measurements were not conducted between day 0 and day 4 and between day 7 and day 11. The analytical measurements between day 4 and day 7 and between day 11 and day 14 show that the active substance is stable into the test medium. However, positive effects (increase of frond number) were observed in concentrations up to and including 25 mg glyphosate IPA/L. This observation is not in line with the ones of previous study conducted with an other Lemna species (CA 8.2.7/001, [redacted] 2002) in which the parameters of the control and concentrations tested up to 11.7 mg glyphosate IPA/L are in the same range and effects appeared at 24.3 mg IPA/L (60.3% on yield and 32.2% on growth rate based on frond number). Thus, actual exposure is questionable. RMS considered the study as valid (validity criteria met) but not reliable for risk assessment.

Data point	CA 8.2.7/004
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study TLA60871 on the toxicity of Glyphosate 62% IPA-Salt to *Lemna gibba* under static conditions.
Report No	110054-009
Document No	-
Guidelines followed in study	Guideline ASTM E 1415- 91 (June 1991)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Not assessed. Study CA 8.2.7/003 ([redacted] 1999) not reliable (see commenting box of RMS above).

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number:	980909FH
Author:	[redacted]
Substance:	Glyphosate 62% IPA-Salt
Title:	Glyphosate 62% IPA-Salt, aquatic plant toxicity test using *Lemna gibba*
Completion date:	12-Feb-1999
Test guideline(s):	Guideline ASTM E 1415- 91 (June 1991)
GLP:	Yes
Testing facility:	DR. U. NOACK-LABORATORIUM, Sarstedt, Germany
Sponsor:	Feinchemie Schwebda GmbH, Köln, Germany

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study TLA60871 ([redacted] 1999) was statistically evaluated for the effects of Glyphosate 62% IPA-Salt on the organism *Lemna gibba*, as the report only provides 14-day endpoints. According to current test guidelines and EFSA Aquatic Guidance (2013), this study type requires a 7-day endpoint.

The organisms were exposed for 14 days to the following concentrations of Glyphosate 62% IPA-Salt: 6.25, 12.5, 25, 50 and 100 mg test item/L, corresponding to 3.9, 7.8, 15.6, 31.2 and 62.4 mg glyphosate/L. Additionally, a control was tested in parallel. The frond count data for the individual control and treatment group replicates will be used to calculate the ECx values.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effect on yield (frond number), and growth rate (frond number) of the test subjects (EC_{10}, EC_{20} and EC_{50} values), a Probit analysis using linear maximum likelihood regression for yield and growth rate (frond number) analysis was performed. For determination of the no-observed-effect concentration (NOEC), Williams Multiple Sequential t-test Procedure was used (one-sided smaller; p=0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

Endpoints based on biomass cannot be determined, as no data for day 7 is available.

II. RESULTS AND DISCUSSION

A. FINDINGS

The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 (2006) are therefore fulfilled.

The percent recovery of nominal test concentrations between 4 and 7 days are presented below.

Table B.9.2.7-16: Analytical verification of test item between 4 and 7 days

Parameter	**Nominal concentration of glyphosate* [mg a.e./L]**				
	3.9	7.8	15.6	31.2	62.4
	Measured concentration of glyphosate acid equivalent [mg/L] (% of nominal)				
Day 4 new	3.37 (86)	6.72 (86)	12.76 (82)	26.87 (86)	48.67 (78)
	3.21 (82)	6.57 (84)	12.54 (80)	26.33 (84)	53.12 (85)
Day 7 aged	4.2 (108)	8.29 (106)	17 (109)	33.44 (107)	67.08 (108)
	4.42 (113)	8.49 (109)	17.12 (110)	33.91 (109)	66.29 (106)
4 - 7 days Geometric mean	**3.8**	**7.5**	**14.7**	**29.9**	**58.2**

* Test concentrations based on active ingredient glyphosate as stated in the study report.

Analytical recovery of the test item ranged from 78 to 113% of nominal from 4 to 7 days duration. Therefore, calculated endpoints will be based on geometric mean measured concentrations.

The parameters for the logit model are estimated as slope b: 3.42368; intercept a: -4.96199 for yield (frond numbers).

The parameters for the Weibull analysis using linear maximum likelihood regression are estimated as slope b: 4.34953; intercept a: -7.06993 for growth rate (frond numbers).

Statistical parameters for goodness of fit are: $Chi^2(13) = 0.61737$; $p(Chi^2)$: 1.000; $F(1,13) = 32.754$, $p(F) < 0.001$; $R^2 = 0.716$ the EC_{10}, EC_{20} and EC_{50} for yield and $Chi^2(13) = 0.23866$; $p(Chi^2)$: 1.000; $F(1,13) = 101.124$; $p(F) < 0.001$; $R^2 = 0.886$ the EC_{10}, EC_{20} and EC_{50} for growth rate, calculations should therefore be considered valid.

The obtained 7-day EC_{10}, EC_{20} and EC_{50} and NOEC values are presented in the table below. The dose response curve obtained from the analysis of the effect of Glyphosate 62% IPA-Salt on the parameters being analysed of *Lemna gibba* is presented below.

Table B.9.2.7-17: 7-day endpoints for Yield (frond number) and Growth Rate (frond number) based on geometric mean measured concentrations.

7-day endpoints	Glyphosate [mg a.e./L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (Frond number)	14.7	6.42 (3.38 – 9.45)	11.1 (7.16 – 15.9)	28.1 (19.3 – 52.0)
Growth rate (Frond number)	14.7	12.8 (9.59 – 15.8)	19.1 (15.4 – 22.6)	34.8 (29.7 – 41.3)

CI: confidence interval

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid.

III. CONCLUSION

Assessment and conclusion by applicant:
The 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values are calculated for yield (frond number) and growth rate (frond number) based on the geometric mean measured concentrations of glyphosate acid equivalents.

The 7-day endpoints of EC_{10}, EC_{20} and EC_{50} values were estimated to be 6.42, 11.1, and 28.1 mg a.e./L for yield (frond number), and 12.8, 19.1, and 34.8 mg a.e./L for growth rate (frond number), respectively.

The statistical parameters presented showed that these values can be considered reliable and therefore considered for risk assessment.

Assessment and conclusion by RMS:
As stated above, RMS considers that the lack of analytical measurements at the beginning and in the middle of the test represent too much uncertainties to consider this study reliable. Therefore, the statistical re-analysis was not assessed.

Data point:	CA 8.2.7/005
Report author	[redacted]
Report year	1996
Report title	Glyphosate acid: Toxicity to duckweed (*Lemna gibba*)
Report No	AB0503/L
Document No	-
Guidelines followed in study	EPA FIFRA Subdivision J Guideline 123-2
Deviations from current test guideline	Deviations from the guideline OECD 221 (2006): Minor: - The study was performed for 14 days instead of 7.
Previous evaluation	Yes, accepted in RAR (2015).
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of Glyphosate acid on growth of *Lemna gibba* were evaluated in a 14 day semi-static toxicity test. The test was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.e./L and a control with 3 replicates per test concentration using three plants per replicate (four fronds each).

The number of fronds affected was determined after 2, 5, 7, 9, 12 and 14 days. Observation of toxicity symptoms were recorded on these dates, too. Sampling and analysis of the test concentration were carried out at test start and on day 5, 9 and 14.
Result showed a significant inhibition of frond number growth of *Lemna gibba* at nominal concentrations of 6.00 mg a.e./L and significant tissue weight inhibition at 12.0 mg a.e./L.
The author concluded that glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.e./L. The 14-d EC_{50} value for inhibition of front number was 12 mg a.e./L (95% CL= 11- 14) and for tissue dry weight 20 mg a.e./L (95% CL= 18 – 22). The NOEC was determined to be 3.0 and 6.0 mg a.e./L for frond number and weight increase, respectively.
The applicant performed a statistical re-analysis of endpoints and based on the mean measured concentration of glyphosate acid the endpoints for 7-day EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 10.5, 14.2 and 24.0 mg a.e./L for yield (frond number), respectively; 13.3, 18.7 and 36.0 mg a.e./L for growth rate (frond number), respectively.
RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%

2. Test organism:

Species:	*Lemna gibba*, Strain G3
Source:	In-house culture originally obtained from University of Waterloo, Canada

3. Environmental conditions:

Temperature:	24.6 – 25.0°C
Photoperiod:	24 h illumination
Light intensity	5000 lux
pH:	Freshly prepared test media: 3.6 – 4.7
	Old test media: 3.6 – 5.8
4. Dates of experimental work:	17th Jan to 31st Jan 1996

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Lemna gibba* was performed with eight concentration levels, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.e./L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions as the test groups.
The plants were placed in 400 mL test vessels, which already contained 160 mL of Hoagland's M-medium prepared according to Hillman (1961). The test was conducted under semi-static conditions with renewal of the test medium after 5 and 9 days. Three uniform healthy-looking plants with 4 fronds each were used in each test vessel.

2. Observations: The number of plants and fronds were counted after 2, 5, 7, 9, 12 and 14 days. Also symptoms of toxicity (*eg*. pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface) were recorded on these dates. At test end the weight of the dried plant tissue (at 60 °C) was recorded. The pH was measured in the old and the new test medium (new= day 0, 5 and 9, old = day 5, 9 and 14). Temperature in the test chamber was recorded daily and light intensity once a week.
Analytical control measurements of the actual concentration of the test item were performed by means of HPLC analysis at test start and after 5 and 9 d (after test medium renewal).

3. Statistical calculations: The EC_{50} and its 95% confidence interval were calculated by moving average angle method using Stephan's method. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at $p = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

The 14-d EC_{50}, NOEC and LOEC values are given below based on nominal concentrations.

Table B.9.2.7-18: Toxicity of Glyphosate acid to *Lemna gibba*

Endpoint	**Glyphosate acid [mg/L]**		
	Frond number	**Biomass dry weigh**	**Visual observed effects**
14-d EC_{50} (95% CL)	12 (11 – 14)	20 (18 – 22)	-
NOEC	3.0	6.0	1.5
LOEC	6.0	12	-

Analytical data: Analytical control measurements were performed in the freshly prepared (day 0, 5 and 9) and the old (day 5, 9 and 14) test media. The measured concentrations of glyphosate acid in the fresh media ranged from 90 – 108% of nominal and in the old media from 87 – 102% of nominal (overall mean measured: 93 – 100% of nominal). As the mean measured content of the glyphosate acid always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the glyphosate acid.

Table B.9.2.7-19: Analytical results

Day sample taken	Nominal concentration of glyphosate acid [mg/L]							
	0.75	**1.5**	**3.0**	**6.0**	**12**	**24**	**48**	**96**
	Measured concentration of glyphosate acid [mg/L]							
0 (fresh)	0.68	1.4	2.9	5.6	12	23	46	92
5 (spent)	0.65	1.3	2.8	5.5	12	24	49	96
5 (fresh)	0.65	1.4	2.8	5.4	12	22	48	92
9 (fresh)	0.75	1.5	3.0	6.0	13	25	50	100
14 (spent)	0.75	1.4	2.9	5.6	12	23	47	98
Mean measured [mg/L]	0.70	1.4	2.9	5.6	12	23	48	96
% of nominal	93	93	97	93	100	96	100	100

B. OBSERVATIONS

The increase in frond number was significantly inhibited starting with a nominal test concentration of 6.0 mg a.e./L when compared to the control. The growth of the plant tissues dry weight was significantly reduced at 12 mg a.e./L. At 24, 48 and 96 mg a.e./L dose related symptoms like pale frond colouration, emergence of stunted new frond growth, reduced root growth and unnatural floating on the solution surface were observed from day 2 onwards. Visually observed effects were apparent at concentrations of 3.0 mg/L and above. Therefore, overall NOEC is 1.5 mg a.e./L.

Table B.9.2.7-20: Frond numbers, increase in frond numbers and inhibition compared to the control

Test item rate [mg/L]	Number of fronds						Increase in frond numbers	Inhibition [%]
	Day 2	**Day 5**	**Day 7**	**Day 9**	**Day 12**	**Day 14**	**(Day 0 – 14)**	
Control	21	48	85	134	222	327	315	-
0.75	23	47	79	125	232	343	331	0
1.5	23	45	78	113	220	323	311	1
3.0	21	48	78	120	206	300	288	9
6.0	21	49	81	116	198	269	257	18*
12	20	44	74	105	148	173	161	49*
24	16	28	44	59	82	91	79	75*
48	15	21	24	28	28	30	18	94*
96	13	14	15	16	18	17	5	98*

*significant inhibition compared to the control medium

Table B.9.2.7-21: Mean dry weight of plant tissue after 14 d, main increase in dry weight and inhibition compared to the control

Test item rate [mg/L]	Mean tissue dry weight after 14 day [mg]	Mean increase [mg]	Inhibition [%]
Control	40.7	39.2	-
0.75	51.3	49.8	0
1.5	49.8	48.3	0
3.0	44.0	42.5	0
6.0	40.3	38.8	1
12	29.8	28.3	28*
24	16.5	15.0	62*
48	6.0	4.5	89*
96	1.4	< 0.1	100*

*significant inhibition compared to the control medium

All validity criteria according to OECD 221 were fulfilled, as the doubling time of frond number in the control were less than 2.4 d.

III. CONCLUSIONS

Assessment and conclusion by applicant:

Glyphosate acid was found to significantly inhibit the growth of *Lemna gibba* after 14 days at or above a nominal concentration of 6 mg a.e./L. The 14-d EC_{50} value for inhibition of front number was 12 mg a.e./L (95% CL= 11- 14 mg test item/L) and for tissue dry weight 20 mg a.e./L (95% CL= 18 – 22 mg a.e./L). The 14-d NOEC was determined to be 3.0 and 6.0 mg a.e./L for frond number and weight increase, respectively.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints. Details of statistical re-evaluation are given in the position paper CA 8.2.7/006.

The 7 day ECx values for yield and growth rate based on frond numbers has been calculated based on nominal concentrations and are provided the table below.

Table B.9.2.7-22: 7-d ECx values for Yield and Growth Rate

7-day endpoints	Concentration of glyphosate acid [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield Frond number	6.0	10.5 (6.76-13.4)	14.2 (10.5-17.1)	24.0 (20.6-27.5)
Growth rate Frond number	12.0	13.3 (10.6-16.7)	18.7 (15.1-23.3)	36.0 (27.5-46.8)

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The doubling time of frond number in the control is slightly above 2.5 days at 14 days (2.94 d) and below 2.5 days for 7 days (2.48 d). Nevertheless, the study is still considered valid by RMS as it is very close to the limit and the validity criterion was fulfilled at 7 days.

The statistical analysis of the endpoints conducted by applicant is presented thereafter. Effects on biomass dry weight could not be calculated for 7 days as they were only measured at 14 days. For completeness, RMS checked effects on growth rates on frond number and on biomass dry weight at 14 days:

Effects on growth rate based on frond number at 14 days

Test item rate (nominal)	Mean Frond number		Mean Growth rate (day 0- 14)	Inhibition (%)
(mg/L)	Day 0	Day 14		
0	12	327	0.236	-
0.75	12	343	0.239	-1.45
1.5	12	323	0.235	0.37
3	12	300	0.230	2.61
6	12	269	0.222	5.91
12	12	173	0.191	19.26
24	12	91	0.145	38.70
48	12	30	0.065	72.28
96	12	17	0.025	89.46

Effects on growth rate based on biomass dry weight at 14 days.

Test item rate (nominal)	Mean tissue dry weight (mg)		Mean Growth rate (day 0- 14)	Inhibition (%)
(mg/L)	Day 0	Day 14		
0	1.5	40.7	0.236	-
0.75	1.5	51.3	0.252	-7.01
1.5	1.5	49.8	0.250	-6.11
3	1.5	44	0.241	-2.36
6	1.5	40.3	0.235	0.30
12	1.5	29.8	0.214	9.44
24	1.5	16.5	0.171	27.35
48	1.5	6	0.099	58.00
96	1.5	1.4	-0.005	102.09

The effects observed on growth rates based on frond number and biomass dry weight at 14 days are in the same range as at 7 days for frond number. Therefore, 14d-ECx values are expected to be covered by those calculated for day 7 based on frond number. In addition, as the validity criterion at 14 days was slightly above 2.5 days, RMS considers ECx values calculated for day 7 more suitable for risk assessment.

Phytotoxicty has been observed but effects were not reported in details in raw data, just global descriptions as presented in the study summaries. Therefore, EC50 based on visual effects cannot be calculated. However, it was noted that the effects were found to be dose related for the three highest dose. The study report indicated that apparent effects were observed at 3.0 mg/L and above. Thus the study authors proposed an NOEC at 1.5 mg/L to consider phytotoxic effects. This should be considered when setting the overall NOEC.

RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.7/006
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study BL5662/B on the toxicity of Glyphosate acid to *Lemna gibba* under static conditions
Report No	110054-010
Document No	-
Guidelines followed in study	EPA FIFRA Subdivision J Guideline 123-2
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

Summary

A statistical evaluation addressing the calculation of valid 7-day EC_{10}, EC_{20}, EC_{50}, and NOEC values was conducted for the study BL5662/B ([redacted] 1996) to fulfill the data requirements according to regulation EU 283/2013. Futhermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).
Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met, this study is considered valid for risk assessment purposes.
Based on the mean measured concentration of glyphosate acid the endpoints for 7-day EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 10.5, 14.2 and 24.0 mg a.e./L for yield (frond number), respectively; 13.3, 18.7 and 36.0 mg a.e./L for growth rate (frond number), respectively.
7d NOErC and 7d NOEyC were 12 and 6 mg glyphosate acide/L (frond number) respectively and a NOEC of 1.5 mg glyphosate acid/L based on phytotoxicity effects was proposed by the authors and RMS.

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number:	AB0503/L
Author:	[redacted]
Substance:	Glyphosate acid
Title:	Glyphosate acid: Toxicity to duckweed (*Lemna gibba*)
Completion date:	31-Jan-1996
Test guideline(s):	EPA FIFRA Subdivision J Guideline 123-2
GLP:	Yes
Testing facility:	Brixham Environmental Laboratory, Zeneca Limited, Brixham Devon, UK
Sponsor:	Zeneca Agrochemicals, Surrey, UK

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study BL5662/B ([redacted] 1996) was statistically evaluated for the effects of Glyphosate on the organism *Lemna gibba* G3 as the report only provides 14 day endpoints. According to current test guidelines and EFSA Aquatic Guidance (2013), this study type requires a 7-day endpoint.

The organisms were exposed for 14 days to the following concentrations of Glyphosate acid: 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.e./L. Additionally, a control was tested in parallel. The frond count data for the individual control and treatment group replicates will be used to calculate the ECx values.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effects on yield (frond number), growth rate (frond number), growth rate (frond area), and growth rate (biomass) of the test subjects (EC_{10}, EC_{20} and EC_{50} values), a non-linear regression model the 3-parameter logistic CDF analysis for yield and the 3-parameter normal CDF growth rate (frond number) analysis was performed.

For determination of the no-observed-effect concentration (NOEC), Williams Multiple Sequential t-test Procedure was used (one-sided smaller; α=0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

Endpoints based on biomass cannot be determined, as no data for day 7 is available.

II. RESULTS AND DISCUSSION

A. FINDINGS

Results
The doubling time of frond numbers in the control was less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days. The validity criteria according to the current guideline OECD 221 (2006) are therefore fulfilled.

The percent recovery of nominal test concentrations between 4 and 7 days are presented below.

Table B.9.2.7-23: Analytical results

Day sample taken	Nominal concentration of glyphosate acid [mg/L]							
	0.75	**1.5**	**3.0**	**6.0**	**12**	**24**	**48**	**96**
	Measured concentration of glyphosate acid [mg/L]							
0 (fresh)	0.68	1.4	2.9	5.6	12	23	46	92
5 (spent)	0.65	1.3	2.8	5.5	12	24	49	96
5 (fresh)	0.65	1.4	2.8	5.4	12	22	48	92
9 (fresh)	0.75	1.5	3.0	6.0	13	25	50	100
14 (spent)	0.75	1.4	2.9	5.6	12	23	47	98
Mean measured [mg/L]	0.70	1.4	2.9	5.6	12	23	48	96
% of nominal	93	93	97	93	100	96	100	100

The measured concentrations of glyphosate acid in the fresh media ranged from 90 – 108% of nominal and in the old media from 87 – 102% of nominal (overall mean measured: 93 – 100% of nominal). As the mean measured content of glyphosate acid always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations.

Table B.9.2.7-24: Frond numbers, growth rates of frond numbers and inhibition compared to the control

Test item rate	**Mean Frond number**		**Mean Growth rate**	**Inhibition**
(mg/L)	**Day 0**	**Day 7**	**(day 0- 7)**	**(%)**
0	12	85	0.280	-
0.75	12	79	0.268	4.2
1.5	12	78	0.266	4.9
3	12	78	0.267	4.6
6	12	81	0.272	2.8
12	12	74	0.259	7.6
24	12	44	0.184	34.1
48	12	24	0.097	65.4
96	12	15	0.032	88.7

The parameters for the 3 parameter logistic CDF model are estimated as b0: 68.792, b1: 23.999 and b2: 2.653 for yield. According to the statistical parameters F (2, 6) = 218.135; p(F) = <0.001; R^2 = 0.986 the EC_{10}, EC_{20} and EC_{50} for yield (frond number) calculations should be considered valid.
The parameters for the 3 parameter normal CDF model are estimated as b0: 0.272, b1: 1.124, and b2: 0.338 for growth rate. According to the statistical parameters; F (2, 6) = 456.502; p(F) = <0.001; R^2 = 0.985 for growth rate the EC_{10}, EC_{20} and EC_{50} calculations should be considered valid.
After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.605 for yield and 0.799 for growth rate.
The 7-day EC_{10}, EC_{20} and EC_{50} values obtained from the analysis of the effect of Glyphosate acid on the parameters being analysed of *Lemna gibba* are presented in the table below.

Table B.9.2.7-25: 7-day EC_x values for Yield and Growth Rate

7-day endpoints	**Concentration of glyphosate acid [mg/L]**			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (frond number)	6.0	10.5 (6.76-13.4)	14.2 (10.5-17.1)	24.0 (20.6-27.5)
Growth rate Frond number	12.0	13.3 (10.6-16.7)	18.7 (15.1-23.3)	36.0 (27.5-46.8)

CI: confidence interval

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid.

III. CONCLUSION

Assessment and conclusion by applicant:
The 7-day EC_{10}, EC_{20} and EC_{50} values are calculated for yield (frond number) and growth rate (frond number) based on the nominal concentration of glyphosate acid.

Based on the mean measured concentration of glyphosate acid the endpoints for 7-day EC_{10}, EC_{20} and EC_{50} values were calculated as follows: 10.5, 14.2 and 24.0 mg a.e./L for yield (frond number), respectively; 13.3, 18.7 and 36.0 mg a.e./L for growth rate (frond number), respectively.

The statistical parameters presented above showed that these values can be considered reliable and therefore considered for risk assessment purposes.

Assessment and conclusion by RMS:
The statistical analysis is considered valid. RMS agrees with ECx calculations. The endpoints retained for the study of [redacted] (1996 CA 8.2.7/005) are as follows:

Frond number

7d NOErC = 12 mg glyphosate acid/L (nom)
7d ErC10 = 13.3 mg glyphosate acid/L (nom)
7d ErC20 = 18.7 mg glyphosate acid/L (nom)
7d ErC50 = 36.0 mg glyphosate acid /L (nom)

7d NOEyC = 6 mg glyphosate acid/L (nom)
7d EyC10 = 10.5 mg glyphosate acid /L (nom)
7d EyC20 = 14.2 mg glyphosate acid /L (nom)
7d EyC50 = 24.0 mg glyphosate acid /L (nom)

The study report of [redacted] (1996 CA 8.2.7/005) indicated that apparent effects were observed at 3.0 mg/L and above. Thus the study authors proposed an NOEC at 1.5 mg/L to consider phytotox effects. Therefore, a NOEC of 1.5 mg glyphosate acid/L based on phytotoxicity effects was proposed.

Data point:	CA 8.2.7/007
Report author	[redacted]
Report year	1987
Report title	The Toxicity of Glyphosate Technical to *Lemna gibba*
Report No	1092-02-1100-5
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Deviations from guideline OECD 221 (2006): Minor: - The study was performed for 14 days instead of 7. - Dry weights are not reported
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary
The effects of glyphosate technical on growth of *Lemna gibba* were evaluated in a 14 day static toxicity test. The definitive test was performed with five concentration levels, encompassing 5, 9, 16, 28 and 50 mg glyphosate/L (measured: 4.28, 9.02, 16.6, 29.0 and 49.4 mg glyphosate/L) in triplicates. Furthermore, a control group with *Lemna gibba* exposed to test medium, without test substance (negative control) was tested.

Three 4-frond colonies and one 3-frond colony, taken from 7-day old stock cultures were aseptically added to 200 mL test medium for a total of 15 fronds per vessel. The pH of the test medium was adjusted prior to the test. Frond counts were made on day 0, 2, 4, 7, 9, 11 and 14 after test initiation. Every frond visibly projecting beyond the edge of the parent frond was counted. The temperature was measured daily and the pH was adjusted to 7.5 ±0.1 at test initiation.

As results, the effects of the test item on frond growth inhibition on day 14, relative to the control, ranged from 14.2% for the measured test concentration of 16.6 mg glyphosate/L to 85.6% for the highest measured test concentration of 49.4 mg glyphosate/L. At or below the measured test concentration of 9.02 mg glyphosate/L, no inhibition effects of the test item on frond's development were observed. All validity criteria according to the OECD guideline 221 were fulfilled.

Analytical recovery of the test item ranged from 99 to 104% on day 0 and from 71 to 104% on day 14. Therefore, calculated endpoints will be based on geometric mean measured concentrations.
The applicant performed a statistical re-analysis of endpoints.The calculated EC_{10}, EC_{20} and EC_{50} values are 18.2, 20.3, and 25.0 mg a.e./L, respectively for yield (frond number) and 20.8, 31.9, and 66.2 mg a.e./L for growth rate (frond number).
RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis study.

The statistical parameters presented showed that these values can be considered reliable and therefore considered for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate
Description:	White solid
Lot/Batch #:	NBP-3594465
Purity:	96.6%
Water solubility	1.2% at 25°C

2. Test organism:

Species:	*Lemna gibba* G3
Source:	In-house culture

3. Environmental conditions:

Temperature:	25 ± 2°C
Photoperiod:	24 h florescence light
Light intensity	4198 - 5813 Lux
pH:	7.5 ± 0.1
Conductivity:	Not stated
Hardness:	Not stated

4. Dates of experimental works: March 30th to April 13th 1987

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed with five concentration levels, 5, 9, 16, 28 and 50 mg glyphosate/L (prepared using 20X-AAP medium), with 3 replicates per test concentration. Furthermore, a control group with *Lemna gibba* exposed to test medium (without test substance) was tested in three replicates under the same conditions as the test groups. Three 4-frond colonies and one 3-frond colony, taken from 7-day old stock cultures were aseptically added to each test vessel, for a total of 15 fronds per vessel. The plants were placed in 1000 mL test vessels, which already contained the 200 mL test media. The pH of the test medium was adjusted prior to the test. The test was conducted under static conditions.

2. Observations: Frond counts were made on day 0, 2, 4, 7, 9, 11 and 14 after test initiation. In order to eliminate subjective decisions on frond maturity, every frond visibly projecting beyond the edge of the parent frond was counted. Fronds were not removed from the test vessels for counting. For each nominal test concentration, the mean measured value on day 0 and day 14 was calculated, based on mean measured test concentrations. Mean frond count values at test termination for each test concentration were expressed as a percent relative to that in the control. On the basis of the mean frond count values, the percentage inhibition was determined and the EC_x values calculated by inverse estimation least squares linear regression. The temperature was measured daily and the pH was adjusted to 7.5 ±0.1 at test initiation. Samples of test media were made at test initiation and test termination for analysis of the active ingredient content in initial and aged test solutions. Samples were analyzed for active substance using HPLC.

3. Statistical calculations: To determine the EC_x values, the log of measured test concentration was plotted against percent inhibition expressed as probit. Inverse estimation least squares linear regression was used to determine the line of best fit and the concentrations corresponding to 25 and 50 percent inhibition and the associated 95% confidence limits were calculated. Parameters of the regression line were determined using the SAS statistical package.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value is given below based on mean measured concentrations.

Table B.9.2.7-26: Toxicity of glyphosate technical to *Lemna gibba*

Endpoint	mg glyphosate/L
EC_{25} (14 day)	18.0
EC_{50} (14 day)	25.5

Analytical data: Chemical analyses were performed on samples of the test solutions to quantify glyphosate in the test solution. The mean measured concentrations were 4.28, 9.02, 16.6, 29.0 and 49.4 mg glyphosate/L, corresponding to 85.6%, 100.2%, 103.8%, 103.6% and 98.8% of the nominal test concentrations, respectively. The mean measured content of the test item always ranged between 80 and 120% of nominal. Nevertheless, the ecotoxicological endpoints were evaluated using mean measured concentrations of the test item.

Table B.9.2.7-27: Analytical results

Parameter	Nominal concentration of glyphosate [mg/L]					
	0	**5**	**9**	**16**	**28**	**50**
	Measured concentration of glyphosate [mg/L]					
Day 0 Concentration	< 0.05	5.01	9.35	16.8	28.8	49.5
Day 14 Concentration	< 0.05	3.54	8.69	16.5	29.1	49.4
Mean measured [mg/L]	< 0.05	4.28	9.02	16.6	29.0	49.4
% of nominal	-	85.6	100.2	103.7	103.6	98.8

Table B.9.2.7-28: Frond counts during assay

Mean Measured Concentration, mg/L[1]		Day 2 4-1-87	Day 4 4-3-87	Day 7 4-6-87	Day 9 4-8-87	Day 11 4-10-87	Day 14 4-13-87
<0.05	A	32	61	170	309	425	656
(0)	B	31	62	172	267	485	689
	C	30	71	164	282	416	649
	Mean	31	65	169	286	442	665
	SD[2]	1.00E+00	5.51E+00	4.16E+00	2.13E+01	3.75E+01	2.14E+01
	Var[3]	1.00E+00	3.03E+01	1.73E+01	4.53E+02	1.41E+03	4.56E+02
4.28	A	31	66	195	333	521	681
(5)	B	29	65	173	268	499	655
	C	28	61	176	331	631	693
	Mean	29	64	181	311	550	676
	SD	1.53E+00	2.65E+00	1.19E+01	3.70E+01	7.07E+01	1.94E+01
	Var	2.33E+00	7.00E+00	1.42E+02	1.37E+03	5.00E+03	3.77E+02
9.02	A	27	57	192	299	525	728
(9)	B	33	73	187	375	569	688
	C	25	54	168	301	513	648
	Mean	28	61	182	325	536	688
	SD	4.16E+00	1.02E+01	1.27E+01	4.33E+01	2.95E+01	4.00E+01
	Var	1.73E+01	1.04E+02	1.60E+02	1.88E+03	8.69E+02	1.60E+03
16.6	A	27	60	167	298	450	549
(16)	B	31	61	173	271	479	586
	C	27	62	177	255	510	582
	Mean	28	61	172	275	480	572
	SD	2.31E+00	1.00E+00	5.03E+00	2.17E+01	3.00E+01	2.03E+01
	Var	5.33E+00	1.00E+00	2.53E+01	4.72E+02	9.00E+02	4.12E+02
29.0	A	25	48	107	143	175	170
(28)	B	26	44	98	145	175	165
	C	23	46	110	153	186	189
	Mean	25	46	105	147	179	175
	SD	1.53E+00	2.00E+00	6.24E+00	5.29E+00	6.35E+00	1.27E+01
	Var	2.33E+00	4.00E+00	3.90E+01	2.80E+01	4.03E+01	1.60E+02
49.4	A	21	36	83	113	115	110
(50)	B	24	40	82	118	125	108
	C	24	33	66	100	100	107
	Mean	23	36	77	110	113	108
	SD	1.73E+00	3.51E+00	9.54E+00	9.29E+00	1.26E+01	1.53E+00
	Var	3.00E+00	1.23E+01	9.10E+01	8.63E+01	1.58E+02	2.33E+00

B. OBSERVATIONS

The effects of the test item on frond growth inhibition on day 14, relative to the control, ranged from 14.2% for the measured test concentration of 16.6 mg glyphosate/L to 85.6% for the highest measured test concentration of 49.4 mg glyphosate/L. At or below the measured test concentration of 9.02 mg glyphosate/L, no inhibition effects of the test item on frond's development were observed.

Table B.9.2.7-29: Percentage growth inhibition of *Lemna gibba* exposed to glyphosate for 14 days

Nominal concentrations [mg glyphosate/L]	**Control**	**5**	**9**	**16**	**28**	**50**
Measured concentrations [mg glyphosate/L]	**-**	**4.28**	**9.02**	**16.6**	**29.0**	**49.4**
Mean number of fronds on Day 7	169	181	182	172	105	77
Mean number of fronds on Day 14	665	676	688	572	175	108
Mean inhibition (14 days) [%]	-	-1.8	-3.6	14.2	75.4	85.6

The doubling time of frond number in the control was less than 2.5 days (2.1 fold in 2 days in the test), and the frond number in the control was more than seven-fold after seven days (approx. 11.3 folds in 7 days in the test). The validity criteria according to guideline OECD 221 are therefore fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 14-day EC_{50} for *Lemna gibba* exposed to glyphosate technical was calculated to be 25.5 mg/L.

Statistical re-analysis of endpoints has been performed to comply with Commission Regulation (EU) 283/2013 to determine 7-day EC_{10}, EC_{20} and EC_{50} endpoints.

The percent recovery nominal test concentrations are presented below.

Table B.9.2.7-30: Analytical verification of test item

Parameter	Nominal concentration of glyphosate [mg/L]					
	0	**5**	**9**	**16**	**28**	**50**
	Measured concentration of glyphosate [mg/L]					
Day 0 Concentration	< 0.05	5.01	9.35	16.8	28.8	49.5
Day 0 % of nominal	**-**	**100**	**104**	**105**	**103**	**99**
Day 14 Concentration	< 0.05	3.54	8.69	16.5	29.1	49.4
Day 14 % of nominal	**-**	**71**	**97**	**103**	**104**	**99**
Geometric mean [mg/L]	**-**	**4.2**	**9.0**	**16.6**	**28.9**	**49.4**

Analytical recovery of the test item ranged from 99 to 104% on day 0 and from 71 to 104% on day 14. Therefore, calculated endpoints will be based on geometric mean measured concentrations.

Details of statistical re-evaluation are given in the position paper CA 8.2.7/008.

The 7 day ECx values for yield and growth rate based on frond numbers has been calculated based on the geometric mean concentrations and are provided in the table below:

Table B.9.2.7-31: 7-d ECx values for Yield and Growth Rate

7-day endpoints	Geometric mean concentration of glyphosate acid [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield Frond number	16.6	18.2 (15.3 – 21.5)	20.3 (17.3 – 23.7)	25.0 (20.7 – 30.2)
Growth rate Frond number	16.6	20.8 (10.9 – 28.9)	31.9 (21.0 – 40.4)	66.2 (55.0 – 77.7)

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

The doubling time of frond number in the control is slightly above 2.5 days at 14 days (2.56 d) and below 2.5 days for 7 days (2.00 d). Nevertheless, the study is still considered valid by RMS as it is very close to the limit and the validity criterion was fulfilled at 7 days. The statistical analysis of the endpoints conducted by applicant is accepted by RMS.
RMS noted that visual observations for signs of phytotoxicity was not available for this study.
RMS overall conclusion regarding the endpoints are presented after the statistical re-analysis below.

Data point	CA 8.2.7/008
Report author	[redacted]
Report year	2020
Report title	Statistical evaluation (non-GLP) of the study 1092-02-1100-5 on the toxicity of Glyphosate to *Lemna gibba* under static conditions
Report No	110054-011
Document No	-
Guidelines followed in study	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
Deviations from current test guideline	Not applicable
Previous evaluation	No, not previously submitted.
GLP/Officially recognised testing facilities	No, GLP is not compulsory for statistical evaluation
Acceptability/Reliability (RMS)	Valid

Summary

A statistical evaluation addressing the calculation of valid 7-day EC_{10}, EC_{20}, EC_{50}, and NOEC values was conducted for the study 1092-02-1100-5 ([redacted] 1987) to fulfil the data requirements according to regulation EU 283/2013. Futhermore, the validity criteria for the study were re- evaluated according to the current guideline OECD 221 (2006).
Analyses were performed using software ToxRatPro Version 3.3.0. The validity criteria according to the current guideline OECD 221 (2006) were met, this study is considered valid for risk assessment purposes.

The calculated EC_{10}, EC_{20} and EC_{50} values are 18.2, 20.3, and 25.0 mg a.e./L, respectively for yield (frond number) and 20.8, 31.9, and 66.2 mg a.e./L for growth rate (frond number).
7d NOErC and 7d NOEyC were 16.6 mg glyphosate acide/L (frond number).

I. MATERIALS AND METHODS

A. MATERIALS

Software: ToxRatPro Version 3.3.0

Original report details

Study number:	1092-02-1100-5
Author:	[redacted]
Substance:	Glyphosate
Title:	The Toxicity of Glyphosate Technical to *Lemna gibba*
Completion date:	13-Apr-1987
Test guideline(s):	Guideline 123-2, U.S. EPA – FIFRA (Growth and Reproduction of Aquatic Plants, Tier 2)
GLP:	Yes
Testing facility:	Malcolm Pirnie, Inc, White Plains, NY 10602, USA
Sponsor:	Monsanto Agricultural Company, Chesterfield, MO 63198, USA

B. STUDY DESIGN

Dates of work: May 2020

Validity of the study was evaluated according to the current test guideline OECD 221 (2006) and 7-day EC_{10}, EC_{20}, EC_{50} and NOEC values were calculated to fulfil the data requirements according to regulation EU 283/2013.

The study 1092-02-1100-5 ([redacted] 1987) was statistically evaluated for the effects of Glyphosate on the organism *Lemna gibba* G3 as the report only provides 14-day endpoints. According to current test guidelines and EFSA Aquatic Guidance (2013), this study type requires a 7-day endpoint.

The organisms were exposed for 14 days to the following concentrations of Glyphosate: 5, 9, 16, 28 and 50 mg glyphosate/L (mean measured: 4.28, 9.02, 16.6, 29.0 and 49.4 mg glyphosate/L). Additionally, a control was tested in parallel. The data used for this evaluation were obtained from original study report.

The analytical data from the study report were checked to ensure the appropriate mean measured or nominal exposure concentrations were used in the ECx calculations.

Statistical calculations

Models providing best fit to the respective data were selected and are as follows:
In order to derive the 7-day Effect Concentrations that have 10, 20 and 50% effects on growth rate and yield of the test subjects (EC10, EC20 and EC50), a Probit analysis using linear maximum likelihood regression for yield (frond number) and a non-linear regression analysis of 3-parameter normal CDF (Cumulative Distribution Function) for growth rate (frond number) was performed. For determination of the no-observed-effect concentration, Williams Multiple Sequential t-test Procedure was used (one-sided smaller; p=0.05).

All statistical evaluations and checks for validity criteria were performed using the software ToxRatPro Version 3.3.0.

Endpoints based on biomass cannot be determined, as no data for day 7 is available.

II. RESULTS AND DISCUSSION

A. FINDINGS

Results
The doubling time of frond number in the control was less than 2.5 days (2.1 fold in 2 days in the test), and the frond number in the control was more than seven-fold after 7 days (approx. 11.3 folds in 7 days in the test). The validity criteria according to guideline OECD 221 are therefore fulfilled.

The percent recovery nominal test concentrations are presented below.

Table B.9.2.7-32: Analytical verification of test item

Parameter	Nominal concentration of glyphosate [mg a.e./L]					
	0	5	9	16	28	50
	Measured concentration of glyphosate [mg a.e./L]					
Day 0 Concentration	< 0.05	5.01	9.35	16.8	28.8	49.5

Day 0 % of nominal	**-**	**100**	**104**	**105**	**103**	**99**
Day 14 Concentration	< 0.05	3.54	8.69	16.5	29.1	49.4
Day 14 % of nominal	**-**	**71**	**97**	**103**	**104**	**99**
Geometric mean [mg/L]	**-**	**4.2**	**9.0**	**16.6**	**28.9**	**49.4**

Analytical recovery of the test item ranged from 99 to 104% on day 0 and from 71 to 104% on day 14. Therefore, calculated endpoints will be based on geometric mean measured concentrations.

Table B.9.2.7-33: Frond numbers, growth rates of frond numbers and inhibition compared to the control

Test item rate	Mean Frond number		Mean Growth rate	Inhibition
(mg/L)	Day 0	Day 7	(day 0- 7)	(%)
0	15	169	0.346	-
5	15	181	0.356	-2.83
9	15	182	0.357	-3.06
16	15	172	0.348	-0.73
28	15	105	0.278	19.65
50	15	77	0.234	32.46

The parameters for the 4 parameter normal CDF model are b0: 162.4, b1: 1.259, b2: 0.109, b3: 61.678 for yield. According to the statistical parameters F (3,2) = 108.669; p(F) <0.001; R^2 = 0.950 the EC_{10}, EC_{20} and EC_{50} calculations for yield (frond number) should be considered valid

For growth rate, the parameters for the 3 parametric logistic CDF model are estimated as b0: 0.357, b1: 66.209, and b2: 1.895. According to the statistical parameters F (3,2) = 79.795; p(F) <0.001; R^2 = 0.919 the EC_{10}, EC_{20} and EC_{50} calculations for growth rate (frond number) should be considered valid. After non-linear regression no lack of fit was detected for the function (p(F|Lack of Fit) = 0.004 for growth rate (frond number).

The obtained EC_{10}, EC_{20} and EC_{50} values are presented in the table below. The dose response curve obtained from the analysis of the effect of Glyphosate on yield (frond number) being analysed of *Lemna gibba* G3 is presented below.

Table B.9.2.7-34: 7-day ECx values for Yield and Growth Rate

7-day endpoints	Geometric mean concentration of glyphosate acid [mg/L]			
	NOEC	EC_{10} (95% CI)	EC_{20} (95% CI)	EC_{50} (95% CI)
Yield (frond number)	16.6	18.2 (15.3 – 21.5)	20.3 (17.3 – 23.7)	25.0 (20.7 – 30.2)
Growth rate Frond number	16.6	20.8 (10.9 – 28.9)	31.9 (21.0 – 40.4)	66.2 (55.0 – 77.7)

CI: confidence interval

III. CONCLUSION

Assessment and conclusion by applicant:
The calculated EC_{10}, EC_{20} and EC_{50} values are 18.2, 20.3, and 25.0 mg a.e./L, respectively for yield (frond number) and 20.8, 31.9, and 66.2 mg a.e./L for growth rate (frond number).

The statistical parameters presented showed that these values can be considered reliable and therefore considered for risk assessment purposes.

Assessment and conclusion by RMS:
The statistical analysis is considered valid.

For completeness, RMS checked effects on growth rates on frond number at 14 days:

Effects on growth rate based on frond number at 14 days

Test item rate (nominal)	Mean Frond number		Mean Growth rate (day 0- 14)	Inhibition (%)
(mg/L)	Day 0	Day 14		
0	15	665	0.271	-
5	15	676	0.272	-0.43
9	15	688	0.273	-0.90
16	15	572	0.260	3.97
28	15	175	0.175	35.2
50	15	108	0.141	47.9

Effects observed at day 14 are higher compared to those observed at day 7. Nevertheless, as 50% effects were not reached neither at day 14 nor at day 7 (for growth rate and inhibition based on the mean number of frond) and as the validity criterion at 14 days was slightly above 2.5 days, RMS considers ECx values calculated for day 7 more suitable for risk assessment.

As visual effects were not measured in the study report, phytotoxic effects could not be taken into account in this study.

Frond number

7d NOErC = 16.6 mg glyphosate acid/L (mm)
7d ErC10 = 20.8 mg glyphosate acid/L (mm)
7d ErC20 = 31.9 mg glyphosate acid/L (mm)
7d ErC50 > 49.4 mg glyphosate acid /L (mm)

7d NOEyC = 16.6 mg glyphosate acid/L (mm)
7d EyC10 = 18.2 mg glyphosate acid /L (mm)
7d EyC20 = 20.3 mg glyphosate acid /L (mm)
7d EyC50 = 25 mg glyphosate acid /L (mm)

Data point	CA 8.2.7/009
Report author	[redacted]
Report year	1987
Report title	The toxicity of glyphosate technical to *Lemna gibba*.
Report No	XX-88-416
Document No	-
Guidelines followed in study	No information mentioned in the Monograph 2001.
GLP	No, GLP was not compulsory at the time the study was performed
Previous evaluation	Not accepted in RAR (2015)
Short description of study design and observations	Toxicity of technical glyphosate (purity >94 %) to aquatic plants (*Lemna gibba*).
Short description of results	No information mentioned in the Monograph 2001.
Reasons for why the study is not considered relevant/reliable or not considered as key study	No study report available and no information mentioned in the Monograph 2001, so these data were considered as not acceptable in the Monograph 2001.
Reasons why the study report is not available for submission (given by applicant)	The notifier does not have access to this study report. Since the study was part of the earlier data package available to the former RMS of the active substance glyphosate, the AGG would have to send a "request for administrative assistance (Art. 39 of Regulation (EC) No. 1107/2009) to the BVL.

RMS notes that the endpoint from this study (reference 1092-02-1100-5) was reported but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
For precautionary reasons, in the absence of study, RMS will consider the endpoint valid for the risk assessment when it is critical.
The endpoint measured in this study (EC50 (14d) = 25.5 mg glyphosate acid./L) is not critical and therefore the study was not reanalysed by RMS.

Data point:	CA 8.2.7/010
Report author	[redacted]
Report year	2012
Report title	Effect of MON77973 (Glyphosate acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment. Test with a subsequent Recovery Period.
Report No	CHE-015/4-80/A
Document No	-
Guidelines followed in study	Maltby, L., *et al.* (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
Deviations from current test guideline	Deviations from guideline: none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Applicant : Valid RMS : Invalid according to OECD guideline 239.

Summary

The toxicity of Glyphosate acid on growth of *Myriophyllum aquaticum* was evaluated in a 14 day static toxicity test, with subsequent 7 day recovery test, performed at concentrations of 5.0, 15.8, 50, 158 and 500 mg glyphosate/L, equivalent to 5.87, 18.5, 58.7, 185.4 and 587 mg glyphosate acid/L. A negative control (Smart & Bako medium) was prepared in parallel.

Two sets of vessels (exposure and recovery set) were prepared, with each set comprising three replicates for each test concentration and six replicates for the controls. Test vessels were 2-L beakers, each containing five individual plants potted in individual pots containing artificial sediment. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days and after 21 days (recovery vessels). At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants. At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. After 14 days, all plants in recovery vessels were transferred to vessels containing dilution water only to assess recovery following exposure.

Test media were analysed for Glyphosate acid content at test start and end of exposure and recovery periods. The measured concentrations ranged from 92.0 – 100.6% of nominal. Glyphosate acid was not detected in the control group.

Relative to the control group, at the highest treatment rate (500 mg glyphosate acid/L) there was 100% growth inhibition based on fresh weight. At 500 mg Glyphosate acid/L fresh weight increase was inhibited by 100%, shoot length increase by 70.8% and growth rate by 57.1%. The recovery period demonstrated that *Myriophyllum aquaticum* pre-exposed to up to 50.0 mg Glyphosate acid/L were able to recover to control levels of growth, in untreated culture medium within 7 days of transfer.

The applicant considered that the study fulfilled the validity criteria of achieving at least 50% increase in control plant growth in terms of length within 7 days of test initiation and therefore concludedthat the test was valid.

RMS checked the validity criteria according to OECD guideline 239 and concluded that the study is not valid.

Glyphosate acid significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a nominal concentration of <5.0 mg glyphosate acid/L. Shoot length was inhibited at or above nominal concentrations of 5.0 mg glyphosate acid/L. The 14-d EC_{50} value for fresh weight inhibition was 12.3 mg glyphosate acid/L and for shoot length it was 78.7 mg glyphosate acid/L. *Myriophyllum aquaticum* pre-exposed for 14 days to up to 50.0 mg glyphosate acid/L were able to recover in untreated culture medium after a 7 day recovery period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate acid (MON77973)
Description:	White crystalline powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	85.2% Glyphosate

2. Test organism:

Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany

3. Environmental conditions:

Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat
	20% kaolin clay
	75-76% quartz sand
	$CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5)
	Based on artificial soil used in OECD guideline 219
	Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	18.0-20.5 °C
Photoperiod:	16 h light/ 8 h dark
Light intensity	6541-7097 lux

pH:	Values recorded at test start and end (in brackets) of 14 day exposure period: Controls = 7.99 (8.14-9.06) 5 mg/L = 8.06 (8.77-10.0) 15.8 mg/L: = 7.99 (8.96-9.96) 50.0 mg/L = 7.36 (7.35-9.13) 158 mg/L = 3.84 (4.88-5.28) 500 mg/L = 2.80 (3.29-3.43) Values at start and end of 7 day recovery period: Recovery period start = 7.95 Recovery period end = 8.17 – 9.48
Oxygen saturation	14 day exposure period: 92 – 94% at the start of the test 114 – 193% at the end of the test 7 day recovery period: 96% at the start of the test 95 – 131% at the end of the test
4. Dates of experimental work:	Sept 27th to Oct 11th 2010

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 5.0, 15.8, 50, 158 and 500 mg glyphosate/L, equivalent to 5.87, 18.5, 58.7, 185.4 and 587 mg Glyphosate acid/L, with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups. Two sets of vessels (exposure and recovery) were prepared at the start of the test

The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate.

After 14 days exposure plants in the recovery set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, were transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event.

2. Observations: Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was recorded at test start and after 14 days.

Analytical control measurements of the actual concentration of the glyphosate acid were performed by means of LC/MS-MS analysis at test start, after 14 (after exposure phase) and 21 days (after recovery phase).

3. Statistical calculations: The EC_{10}, EC_{20} and EC_{50} and its 95% confidence interval were calculated by Probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Williams' t-test, Dunnett's t-test or Welch's t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Analytical control measurements of the actual concentration of the glyphosate acid were performed at test start and after 14 days. The measured concentrations ranged from 92.0 – 100.6% of nominal. As the mean measured content of the test item always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Additional analytical measurements were made at the end of the 7 d recovery period (at day 21). The measured concentration in the test media were < LOQ at the lowest test concentrations and between 1.7 and 2.1% of the test media concentrations at the end of the growth test.

Table B.9.2.7-35: Analytical results

Nominal [mg/L]	Test start 14 d growth test		End of test 14 d growth test		End of 7 d recovery test	
	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal
Control	< LOQ -	-	<LOQ	-	< LOQ	-
5.0	4.95	99.1	4.86	97.2	< LOQ	-
15.8	15.4	97.4	14.5	92.0	0.32	2.1
50.0	49.8	99.6	49.6	99.3	1.03	2.1
158	149	94.3	157	99.2	2.73	1.7
500	488	97.6	503	100.6	8.70	1.7
Pore water 500 mg/L	-	-	95.1	19.0	28.8	5.8

LOQ= 0.25 mg/L

The $EC_{50,}$ EC_{20} and NOEC values after 14 day growth inhibition test are given below based on nominal concentrations.

Table B.9.2.7-36: 14-day endpoints

Endpoint	Glyphosate acid [mg/L]			
	14 Day EC_{10}	14 Day EC_{20}	14 Day EC_{50}	14 Day NOEC
Shoot length/yield	n.d.	4.05 (0.82 - 9.35)*	78.7 (46.1 - 146)	5.0
Shoot length/growth rate	2.40* (0.31-6.76)	12.1 (3.55-24.2)	276 (159 - 664)	5.0
Fresh weight/yield	n.d.	1.72 (0.88 - 2.75)*	12.3 (9.19 - 15.8)	<5.0
Fresh weight/ growth rate	n.d.	3.60 (1.85 - 5.69)*	23.4 (17.2 - 30.9)	<5.0
Dry weight/yield	3.06* (0-10.7)	6.31 (0 - 17.6)	25.2 (2.61 - 151)	50.0
Dry weight/ growth rate	3.68* (0-12.8)	7.58 (0 - 21.1)	30.2 mg/L (3.54-191)	50.0
Root length/yield	n.d.	3.26 *	18.0 (5.19 - 43.0)	<5.0
Root length/growth rate	n.d.	n.d.	>500	<5.0

CI = 95% confidence interval
* extrapolated, lowest test concentration was 5.0 mg/L.
n.d. not determined

The $EC_{50,}$ EC_{20} and NOEC values after 7 day recovery period are given below based on nominal concentrations.

Table B.9.2.7-37: 7-day endpoints for recovery

Endpoint	Glyphosate acid [mg/L]			
	7 Day EC_{10}	**7 Day EC_{20}**	**7 Day EC_{50}**	**7 Day NOEC**
Shoot length/yield	26.0 (14.0-37.1)	41.2 (26.5-54.2)	99.5 (79.7-125)	50
Shoot length/growth rate	29.5 (14.6-43.3)	46.9 (28.5-63.0)	114 (89.5-147)	50
Fresh weight/yield	n.d.	n.d	n.d.	158
Fresh weight/ growth rate	n.d.	n.d	n.d.	158
Dry weight/yield	n.d.	n.d	n.d.	≥500
Dry weight/ growth rate	n.d.	n.d	n.d.	≥500
Root length/yield	>500	>500	>500	≥500
Root length/growth rate	>500	>500	>500	≥500

n.d.: not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS

There was a concentration dependent effect on growth, root length, fresh and dry weight of *Myriophyllum aquaticum.* Growth was significantly reduced at 5.00 mg glyphosate/L, fresh weight at <50 mg Glyphosate acid/L, dry weight at 50.0 mg Glyphosate acid/L and root length at <50 mg Glyphosate acid/L during the 14 day exposure test. In the subsequent recovery test; it was shown that *Myriophyllum aquaticum*, pre-exposed to up to 50.0 mg Glyphosate acid/L were able to recover to control levels of growth in untreated culture medium within 7 days of the exposure period.

Table B.9.2.7-38: Percentage of inhibition of shoot length of *Myriophyllum aquaticum* exposed for 14 days to glyphosate acid

Test parameters	Glyphosate acid [mg/L]				
	5.0	**15.8**	**50.0**	**158**	**500**
Inhibition of shoot length increase (%)	19.2	29.9	55.9	50.3	70.8
Inhibition of shoot length growth rate (%)	11.8	19.5	41.9	36.7	57.9
Inhibition of fresh weight increase (%)	34.2	57.5	69.2	83.7	109
Inhibition of fresh weight growth rate (%)	24.6	46.5	59.0	76.7	115
Inhibition of dry weight increase (%)	-11.8	46.5	26.8	92.7	108
Inhibition of dry weight growth rate (%)	-10.2	40.8	40.4	92.4	114
Inhibition of root length increase (%)	19.4	52.3	76.0	79.7	88.8
Inhibition of root length growth rate (%)	2.0	7.0	13.5	15.1	21.1

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group, with an increase of biomass (shoot length) in controls was > 50%, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The mean measured content of the test item always ranged between 80 and 120% of nominal so the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

The 14-d ErC_{50} value for fresh weight was 23.4 mg a.e./L and for shoot length it was 276 mg a.e./L.

The study is considered valid and reliable for risk assessment purposes.

Endpoint in glyphosate acid	14 Day EC_{50} [mg/L]	14 Day NOEC [mg/L]
Shoot length/yield	78.7	5.0
Shoot length/growth rate	276	5.0
Fresh weight/yield	12.3	<5.0
Fresh weight/ growth rate	**23.4**	<5.0
Dry weight/yield	25.2	50.0
Dry weight/ growth rate	30.2	50.0
Root length/yield	18.0	<5.0
Root length/growth rate	>500	<5.0

Assessment and conclusion by RMS:

RMS checked validity criteria according to OECD Guideline 239. The mean total shoot length and mean total shoot fresh weight in control plants doubled during the exposure phase of the test. However, the coefficient of variation for yield based on measurements of shoot fresh weight in the control cultures exceeded 35% between replicates (42.6%). The test design differed from the guideline in the number of plants per replicate, which was of 5 instead of 3, which may contribute to an unexpected behavior of the control (CV>35%).
The study is not valid.

Based on its fate characteristics, glyphosate is considered as persistant in sediment. Thus exposure of rooted aquatic plants is expected. RMS therefore considered that further information to assess the effects of glyphosate on rooted aquatic macrophytes is required (data gap).

Data point:	CA 8.2.7/011
Report author	[redacted]
Report year	2012
Report title	Effect of AMPA (Aminomethylphosphonic acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment, with a subsequent Recovery Period
Report No	CHE-022/4-80/A
Document No	-
Guidelines followed in study	Maltby, L., et al. (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP
Deviations from current test guideline	Deviations from guideline: none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The toxicity of Glyphosate acid on growth of *Myriophyllum aquaticum* was evaluated in a 14 day static toxicity test, with subsequent 7 day recovery test, performed at concentrations of 1.0, 2.6, 6.4, 16, 40 and 100 mg AMPA/L. A negative control (Smart & Bako medium) was prepared in parallel.

Two sets of vessels (exposure and recovery set) were prepared, with each set comprising three replicates for each test concentration and six replicates for controls were used. Test vessels were 2-L beakers, each containing five individual plants potted in individual pots containing artificial sediment. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days and after 21 days (recovery vessels). At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants. At the end of the test all plants were harvested, and the root length was assessed semi-quantitatively in terms of length of the main root. After 14 days, all plants in recovery vessels were transferred to vessels containing dilution water only to assess recovery following exposure.

Test media were analysed for AMPA content at test start, test end and at the end of the recovery period. The measured concentrations ranged from 75.5 - 102% of nominal. AMPA was not detected in the control group. Therefore; the test was evaluated using the geometric mean measured concentrations.

Result showed a significant inhibition of fresh weight and shoot length at the lowest test concentration of >14.3 mg AMPA/L. The following recovery test demonstrated that *Myriophyllum aquaticum* pre-exposed to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

The study fulfilled the validity criteria of achieving at least 50% increase in control plant growth in terms of length within 7 days of test initiation. The test was therefore considered to be valid.

AMPA significantly inhibited the fresh weight and shoot length of *Myriophyllum aquaticum* after 14 days at a nominal concentration of >14.3 mg AMPA/L. The 14-d EC_{50} value for fresh weight inhibition was 70.8 mg AMPA/L and for shoot length > 94.6 mg AMPA/L. *Myriophyllum aquaticum* pre-exposed

for 14 day to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solids
Lot/Batch #:	GLP-0905-19864A (recertified as GLP-110521446-A)
Purity:	98.5%

2. Test organism:

Species:	*Myriophyllum aquaticum*
Source:	Institut für Gewässerschutz, MESOCOSM GmbH, Neu-Ulrichstein 5, D-35315 Homberg (Ohm), Germany

3. Environmental conditions:

Growth medium:	Smart & Bako medium
Artificial sediment:	4-5% peat
	20% kaolin clay
	75-76% quartz sand
	$CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5)
	Based on artificial soil used in OECD guideline 219
	Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)
Temperature:	20.5 – 21.0 °C
Photoperiod:	16 h light/ 8 h dark
Light intensity	7571 - 7903 lux
pH:	<u>Values recorded at test start and end (in brackets) of 14 day exposure period</u>:
	Controls = 7.91 (8.54–8.91)
	0.88 mg/L = 8.06 (8.04-8.08)
	2.23 mg/L: = 7.99 (8.05-8.11)
	5.43 mg/L = 7.36 (8.05-8.07)
	14.3 mg/L = 3.84 (7.90-7.99)
	37.1 mg/L = 2.80 (7.75-7.79)
	94.6 mg/l = 6.60 (7.23-7.33)
	<u>Values at start and end of 7 day recovery period:</u>
	Recovery period start = 7.97-9.04
	Recovery period end = 8.18 – 9.28
Oxygen saturation	<u>14 day exposure period:</u>

	95 – 97% at the start of the test 101 – 138% at the end of the test <u>7 day recovery period:</u> 96 – 138% at the start of the test 90 – 114% at the end of the test
4. Dates of experimental work:	Aug 18th to Sept 8th 2011

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 1.0, 2.6, 6.4, 16, 40 and 100 mg AMPA/L with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups.

The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate. After 14 days exposure another set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, was transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event.

2. Observations: Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 5, 8 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was at test start and after 14 days.

Analytical control measurements of the actual concentration of AMPA were performed by means of LC/MS-MS analysis at test start, after 14 and 21 days (after recovery phase).

3. Statistical calculations: The EC_{10}, EC_{20} and EC_{50} and its 95% confidence interval were calculated by Probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Dunnett's t-test or Welch's t-test (p = 0.05).

II. RESULTS AND DISCUSSION

A. FINDINGS

<u>Analytical data</u>: Analytical control measurements of the actual concentration of AMPA were performed at test start, after 14 and 21 days (after recovery phase). The measured concentrations ranged from 75.5 - 102% of nominal. Therefore the test was evaluated using the geometric mean measured concentrations.

Measured concentrations of AMPA in the macrophyte growth inhibition test are depicted below.

Table B.9.2.7-39: Analytical results

Nominal [mg/L]	Test start 14 d growth test		End of test 14 d growth test		Mean measured [mg/L]
	Measured [mg/L]	% of nominal	Measured [mg/L]	% of nominal	
Control	< LOQ -	-	<LOQ	-	< LOQ
1.0	1.02	101.7	0.76	76.4	0.88
2.6	2.49	95.8	1.99	76.6	2.23
6.4	6.09	95.2	4.85	75.7	5.43
16	15.5	96.6	13.2	82.2	14.26
40	40.0	100.0	34.4	86.1	37.13
100	98.3	98.3	91.1	91.1	94.61

LOQ = limit of quantification = 0.5 mg/L

The EC_{50} and NOEC values after 14-day growth inhibition test are given below based on geometric mean measured concentrations.

Table B.9.2.7-40: 14-day endpoints

Endpoint	AMPA [mg/L][#]			
	14 Day EC_{10}	14 Day EC_{20}	14 Day EC_{50}	14 Day NOEC
Shoot length/yield	1.3 (0.2-3.2)	5.8 (2.1-10.4)	103.3* (54.8-337)	14.3
Shoot length/growth rate	6.1 (2.2-10.6)	22.5 (13.7-33.1)	> 94.6	14.3
Fresh weight/yield	19.7 (11.3-26.9)	30.6 (21.0-38.3)	70.8 (59.4-87.7)	14.3
Fresh weight/ growth rate	24.2 (14.5-32.2)	39.0 (28.4-47.5)	97.3 (81.8-126)*	14.3
Dry weight/yield	33.9 (17.7-44.9)	42.0 (25.7-53.2)	63.2 (49.0-79.2)	37.1
Dry weight/ growth rate	38.4 (22.2-49.1)	47.6 (31.6-58.1)	72.0 (59.4-83.6)	37.1
Root length/yield	5.1 (4.0-6.2)	9.5 (7.9-11.0)	31.1 (28.1-34.6)	5.4
Root length/growth rate	17.0 (14.9-19.0)	35.9 (33.2-38.5)	150.1*(136.1-168.1)	5.4

* extrapolated, highest test concentration was 94.6 mg AMPA/L
[#] 95% confidence intervals presented in brackets.

The EC_{50} and NOEC values after 7 day recovery period are given below based on geometric mean measured concentrations.

Table B.9.2.7-41: 7-day recovery endpoints

Endpoint	AMPA [mg/L][#]			
	7 Day EC_{10}	**7 Day EC_{20}**	**7 Day EC_{50}**	**7 Day NOEC**
Shoot length/yield	5.4 (0-15.7)	13.5 (0.1-31.1)	78.2 (34.2-6082.1)*	37.1
Shoot length/growth rate	6.4 (0-17.6)	16.0 (0.2-35.3)	92.8 (41.9-8310.6)	37.1
Fresh weight/yield	1.4 (0-4.8)	3.0 (0-8.1)	12.6 (2.5-79.7)	5.4
Fresh weight/ growth rate	1.5 (0-5.1)	3.2 (0-8.7)	13.6 (2.8-87.3)	5.4
Dry weight/yield	n.d.	n.d.	≥ n.d.	≥ 94.6
Dry weight/ growth rate	n.d.	n.d.	n.d.	≥ 94.6
Root lengthyield	n.d.	n.d.	≥ n.d.	≥ 94.6
Root length/growth rate	n.d.	n.d.	n.d.	≥ 94.6

[#] 95% confidence intervals presented in brackets.
n.d.: not determined due to mathematical reasons or inappropriate data

B. OBSERVATIONS

There was a concentration dependent effect on growth, fresh and dry weight of *Myriophyllum aquaticum.* Growth and fresh weight was significantly reduced at >14.3 mg AMPA/L. In the subsequent recovery test it was shown that *Myriophyllum aquaticum*, pre-exposed to up to 5.4 mg AMPA/L were able to recover in untreated culture medium after a 7 day recovery period.

Table B.9.2.7-42: Percentage of inhibition of shoot length of *Myriophyllum aquaticum* exposed for 14 days to AMPA

Test parameters	AMPA [mg/L]					
	0.88	**2.23**	**5.43**	**14.26**	**37.13**	**94.61**
Inhibition of shoot length increase (%)	20.8	16.8	12.5	16.7	40.8	54.3
Inhibition of shoot length growth rate (%)	11.7	9.2	6.4	9.0	26.4	38.0
Inhibition of fresh weight increase (%)	-14.1	-15.2	-7.0	-10.9	29.0	60.2
Inhibition of fresh weight growth rate (%)	-9.0	-9.4	-3.9	-6.9	20.8	48.3
Inhibition of dry weight increase (%)	-47.5	-45.6	-7.1	1.1	-4.6	79.9
Inhibition of dry weight growth rate (%)	-28.9	-26.5	-4.9	1.6	-2.1	71.2
Inhibition of root length increase (%)	-13.1	-8.8	15.7	26.4	55.0	79.3
Inhibition of root length growth rate (%)	-3.5	-2.5	4.2	7.7	20.4	39.5

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group; with an increase of biomass (shoot length) in controls was > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The EC_{50} and NOEC values after 14-day growth inhibition test are given below based on geometric mean measured concentrations.

The 14-d ErC_{50} value for dry weight was 72.0 mg AMPA/L, fresh weight was 97.3 mg AMPA/L and for shoot length > 94.6 mg AMPA/L.

The study is considered valid so the following EC_{50} and NOEC can be used for risk assessment purposes:

Endpoint in AMPA	14 Day EC_{50} [mg/L]	14 Day NOEC [mg/L]
Shoot length/relative increase	103.3*	14.3
Shoot length/growth rate	> 94.6	14.3
Fresh weight/relative increase	70.8	14.3
Fresh weight/ growth rate	97.3	14.3
Dry weight/relative increase	63.2	37.1
Dry weight/ growth rate	**72.0**	37.1
Root length/relative increase	31.1	5.4
Root length/growth rate	150.1*	5.4

* extrapolated, highest test concentration was 94.6 mg AMPA/L

Assessment and conclusion by RMS:

RMS checked validity criteria according to OECD Guideline 239. The mean total shoot length and mean total shoot fresh weight in control plants doubled during the exposure phase of the test and the coefficient of variation for yield based on measurements of shoot fresh weight in the control cultures did not exceed 35% between replicates (12.3%). Thus the study is considered valid.

RMS noted that the test design differed from the guideline OECD 239 in the number of plants per replicate, which was of 5 instead of 3. As the control behave as expected (validity criteria of OECD 239 met), this is not considered to influence the outcome of the study.

There are inconsistencies in the study report about the effects/endpoints on fresh weight summarized at the beginning of the report and those presented later in the data analysis. Nevertheless, RMS checked these effects/endpoints from raw data and confirms that what is summarized above is correct. Moreover, some EC50 values calculated are extrapolated values as they are above the higher tested concentration. EC50 found to be outside the tested range have been set to be greater than the highest tested dose.

Shoot length

14d NOErC = 14.3 mg AMPA/L (mm)
14d ErC10 = 6.1 mg AMPA/L (mm)
14d ErC20 = 22.5 mg AMPA/L (mm)
14d ErC50 > 94.6 mg AMPA/L (mm)

14d NOEyC = 5.43 mg AMPA/L (mm)
14d EyC10 = 1.3 mg AMPA/L (mm)

14d EyC20 = 5.8 mg AMPA/L (mm)
14d EyC50 > 94.6 mg AMPA/L (mm)

Shoot fresh weight

14d NOErC = 14.3 mg AMPA/L (mm)
14d ErC10 = 24.2 mg AMPA/L (mm)
14d ErC20 = 39 mg AMPA/L (mm)
14d ErC50 > 94.6 mg AMPA/L (mm)

14d NOEyC = 14.3 mg AMPA/L (mm)
14d EyC10 = 19.7 mg AMPA/L (mm)
14d EyC20 = 30.6 mg AMPA/L (mm)
14d EyC50 = 70.8 mg AMPA/L (mm)

Shoot dry weight

14d NOErC = 37.1 mg AMPA/L (mm)
14d ErC10 = 38.4 mg AMPA/L (mm)
14d ErC20 = 47.6 mg AMPA/L (mm)
14d ErC50 = 72 mg AMPA/L (mm)

14d NOEyC = 37.1 mg AMPA/L (mm)
14d EyC10 = 33.9 mg AMPA/L (mm)
14d EyC20 = 42 mg AMPA/L (mm)
14d EyC50 = 63.2 mg AMPA/L (mm)

Root length

14d NOErC = 14.3 mg AMPA/L (mm)
14d ErC10 = 17 mg AMPA/L (mm)
14d ErC20 = 35.9 mg AMPA/L (mm)
14d ErC50 > 94.6 mg AMPA/L (mm)

14d NOEyC = 2.23 mg AMPA/L (mm)
14d EyC10 = 5.1 mg AMPA/L (mm)
14d EyC20 = 9.5 mg AMPA/L (mm)
14d EyC50 = 31.1 mg AMPA/L (mm)

A recovery could be expected after 7 days without exposure to active substance for plants exposed up to and including 5.43 mg AMPA/L (mm).

Data point:	CA 8.2.7/012
Report author	[redacted]
Report year	2011
Report title	HMPA (hydroxymethylphosphonic acid): A 7-Day Static-Renewal Toxicity Test with Duckweed (*Lemna gibba* G3)
Report No	139A-397
Document No	-
Guidelines followed in study	OPPTS 850.4400, ASTM Standard Guide 1415-91 E (1991) OECD Guideline 221 (2006)
Deviations from current test guideline	Deviation from guideline OECD 221 (2006): none
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Summary

The effects of HMPA (hydroxymethylphosphonic acid) on growth of *Lemna gibba* G3 were evaluated in a 7-day static-renewal toxicity test at nominal concentrations of 7.5, 15, 30, 60, and 120 mg HMPA/L, corresponding to mean measured concentrations of 7.4, 15, 30, 60 and 123 mg HMPA/L, respectively. A negative control was prepared in parallel. Three replicates were prepared per control and test item treatment using four plants (totalling 12 fronds) per replicate, each. The pH of the 20X AAP test medium was adjusted to 7.6 with 0.1 N NaOH. Renewal of the test media was performed on day 3 after test initiation. Direct counts of number of fronds were conducted on day 3, 5 and 7. Observations of chlorosis, necrosis, break-up of duckweed colonies, root destruction, death and any other abnormalities in plant or frond appearance were also performed at those times. Dry weight was determined at the beginning (representative sample) and at the end of the test (each vessel). EC_{50} values were calculated based on replicate frond counts, biomass and growth rates based on frond counts and biomass on day 7 of the test. Analysis of the test concentration was carried out at test initiation, on day 3 and at test termination on day 7. The mean measured content of the test item ranged between 99 and 103% of nominal concentrations. HMPA was not detected in the control group.

Percent inhibition of frond growth in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -9, -15, -1, -7 and -20%, respectively. Percent inhibition of growth rate based on frond number in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -4, -6, -1, -4, and -8%, respectively. Percent inhibition biomass in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -13, -25, -15, -20 and -33%, respectively. Percent inhibition of growth rate based on biomass in the 7.4, 15, 30, 60 and 123 mg HMPA/L treatment groups at test termination was -5, -9, -6, -8 and -12%, respectively.

Based on these results, the EC_{50} for frond number, biomass and growth rates based on frond number and biomass for HMPA was determined to be >123 mg HMPA/L. After 7 days of exposure, there were no apparent treatment-related effects upon growth at any of the concentrations tested. The validity criteria according to guideline OECD 221 are fulfilled.

Since no inhibition effects of HMPA were observed on frond number, frond number growth rate, biomass and biomass growth rate of *Lemna gibba* after 7 days at all concentrations tested, the EC_{50}

values after 7 days of exposure were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be 123 mg HMPA/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	HMPA (hydroxymethylphosphonic acid)
Description:	Solid
Lot/Batch #:	GLP-1003-20448-A
Purity:	97.0%

2. Test organism:

Species:	*Lemna gibba* G3, up to 7 days old
Source:	In-house culture

3. Environmental conditions:

Temperature:	23.7 – 25.4 °C
Light intensity:	Continuous illumination, 4410 - 5250 lux
pH:	7.1 – 8.0 at test start; 8.8 – 9.0 at test termination
Hardness:	20.88 mg (K_2HPO_4/L)
4. Dates of experimental work	June 10th to June 19th 2010

B. STUDY DESIGN AND METHODS

1. Experimental treatments: On the basis of the results of a range finding test, the definitive test was performed at five concentration levels, 7.5, 15, 30, 60, and 120 mg HMPA/L with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Four plants totalling 12 fronds were added to each replicate test chamber. The plants were placed in 250 mL test vessels containing 100 mL 20X-AAP test media. The pH of the test medium was adjusted with 0.1N NaOH prior to the test. The test was conducted under a 7-day static-renewal test conditions. The renewal of the test media was performed on day 3 after test initiation.

2. Observations:

Biological data: The toxicity of HMPA to duckweed was determined by direct counts of frond numbers and observations for chlorosis, necrosis, dead fronds and frond appearance were made on Days 3, 5 and 7. Dry weight was measured at the beginning of the test on a representative sample from the culture used to initiate the test. At the end of the test, dry weight was determined from each test vessel.

Physical data: The pH values were measured on day 0, 3, and 7. Temperature was measured continuously and recorded twice daily. Samples of the test solutions were collected from new solution of each experimental group at the beginning of the test, from new solutions and pooled old solutions at the end of the renewal period on Day 3, and from pooled test solutions at test termination to determine test substance concentrations. Samples were processed immediately for analysis. All test concentrations and control replicates were analysed using HPLC with mass selective detection.

3. Statistical calculations: The 7-day EC_{50} value for frond counts; biomass and growth rates based on frond counts and biomass are based on descriptive analysis of the data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test for inhibition of frond number and biomass dry weight, respectively, at $\alpha = 0.05$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: In freshly prepared test media the recovery of the active substance ranged between 92.5 % and 103%. In the aged test media (7 days old), 104% to 110% of the active substance was recovered. Samples from new and old test solution at Day 3 renewal ranged from 90.1 to 101% and 96.9 to 107%, respectively. The overall mean measured concentrations were within the range of 80 to 120% of nominal however, the results were based on mean measured concentrations.

Table B.9.2.7-43: Analytical results

Nominal concentration [mg HMPA/L]	**7.5**	**15**	**30**	**60**	**120**
Day 0 concentration (fresh)	7.61	15.3	30.8	56.2	111
Day 3 concentration (spent)	6.89	14.3	27.0	55.3	121
Day 3 concentration (fresh)	7.36	14.5	30.0	64.3	126
Day 7 concentration (spent)	7.84	16.0	32.5	64.8	132
Mean measured [mg HMPA/L]	7.4	15	30	60	123
% of nominal	99	100	100	100	103

The overall mean measured concentrations were within the range of 80 to 120% of nominal however, the results were based on mean measured concentrations.

The EC_{50} and NOEC values are given below based on mean measured concentrations.

Table B.9.2.7-44: Endpoints

Endpoint	**mg HMPA/L**
$EC_{50,\ frond\ number}$ (7 day)	>123
$NOEC_{frond\ number}$ (7 day)	≥123
$EC_{50,\ biomass}$ (7 day)	>123
$NOEC_{\ biomass}$ (7 day)	≥123
$EC_{50,\ growth\ rate\ (frond\ number)}$ (7 day)	>123
$NOEC_{\ growth\ rate\ (frond\ number)}$ (7 day)	≥123
$EC_{50,\ growth\ rate\ (biomass)}$ (7 day)	>123
$NOEC_{growth\ rate\ (biomass)}$(7 day)	≥123

B. OBSERVATIONS

Observations: None of the parameters recorded, i.e. frond number, biomass, growth rate based on front number and growth rate based on biomass was found to be significantly different from the control (Dunnett's t-test [$\alpha = 0.05$]); see the table below.

Table B.9.2.7-45: Frond numbers and inhibition values of *Lemna gibba* G3 after 7 days of exposure to HMPA

Test item	**Control**	**HMPA [mg/L]**				
Nominal concentrations [mg HMPA/L]	-	**7.5**	**15**	**30**	**60**	**120**
Mean measured concentrations [mg HMPA/L]	-	**7.4**	**15**	**30**	**60**	**123**
Mean frond number	145	158	166	147	156	174
Mean inhibition [%]	-	-9	-15	-1	-7	-20
Mean biomass [mg]	16.73	18.90	20.93	19.17	20.10	22.20
Mean inhibition [%]		-13	-25	-15	-20	-33
Mean growth rate based on frond number	0.3531	0.3681	0.3751	0.3564	0.3656	0.3818
Mean inhibition [%]	-	-4	-6	-1	-4	-8
Mean growth rate based on biomass	0.3494	0.3679	0.3821	0.3699	0.3763	0.3909
Mean inhibition [%]	-	-5	-9	-6	-8	-12

The doubling time of frond numbers in the control was less than 2.5 days (1.96 days), corresponding to approximately a twelve-fold increase after seven days. The validity criteria according to the current guideline OECD 221 are therefore fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The EC_{50} and NOEC values are given below based on mean measured concentrations.

Since no inhibition effects of HMPA was observed on the frond number, frond number growth rate, biomass and biomass growth rate of *Lemna gibba* G3 after 7 days at all concentrations tested, the EC_{50} values for frond number, frond number growth rate, biomass and biomass growth rate were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be ≥123 mg HMPA/L.

The EC_{50} values for frond number, frond number growth rate, biomass and biomass growth rate were all >123 mg HMPA/L, the highest concentration tested. The NOEC was determined to be ≥123 mg HMPA/L.

The validity criteria according to the current guideline OECD 221 were met and this study is considered valid for risk assessment purposes.

Assessment and conclusion by RMS:

All validity criteria according to OECD 221 guideline were met. The study is valid.

Endpoints based on frond number and biomass dry weight are reported below:

7d NOECr = 123 mg HMPA/L (nom)
7d ErC10 > 123 mg HMPA/L (nom)

7d ErC20 > 123 mg HMPA/L (nom)
7d ErC50 > 123 mg HMPA/L (nom)

7d NOECy = 123 mg HMPA/L (nom)
7d EyC10 > 123 mg HMPA/L (nom)
7d EyC20 > 123 mg HMPA/L (nom)
7d EyC50 > 123 mg HMPA/L (nom)

Data point:	CA 8.2.7/013
Report author	Yanhui, T.. *et al.*
Report year	2015
Report title	Growth inhibition of two herbicides on *Spirodela polyrhiza*
Document No	ISSN: 1002-5480
Guidelines followed in study	OECD 221
Deviations from current test guideline	Not reported
Previous evaluation	No
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability (RMS):	Applicant : Yes/Reliable with restrictions RMS : Relevant / reliability not assignable (data gap : provide an English certified translation)

For details assessment please refer to Appendix to Volume 3 CA B.9 on general literature review on ecotoxicology

Assessment and conclusion by applicant:

The effects of glyphosate to the aquatic macrophyte *Spirodela polyrhiza* was tested in a semi-static exposure of 7 days at concentrations between 8.4 and 20.902 mg/L. The 7 day-EC_{50} value was determined to be 12.817 mg/L.

This study was conducted to guideline but not to GLP. The test concentrations were not analytically verified and thus the exact exposure concentrations of the aquatic macrophyte are unknown. Therefore, the study should considered as reliable with restrictions.

Assessment and conclusion by RMS:

The study report is in Chinese and a translated version was not available to RMS.
Validity criteria, biomass and growth rates could not be checked as no raw data is presented and only the graphics or tables presented above are available in the study summary.
In addition, the study is not GLP and analytical measurements of nominal concentrations were not conducted.
Therefore, RMS considers this study not reliable for risk assessment.

B.9.2.8. Further testing on aquatic organisms

According to Regulation EU No 283/2014, further studies on aquatic organisms may be conducted to refine the identified risk and shall provide sufficient information and data to evaluate potential impact on aquatic organisms under field conditions.

Such further studies on aquatic organisms have not been submitted. Datagap has been identified in order to finalise the aquatic risk assessment. However data has been proposed to address the risk of indirect effects (see Volume 3 CP B.9 under B.9.14.1.2. Aquatic organisms - Risk to biodiversity via Indirect Effects and Trophic Interactions).

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute toxicity to bees

B.9.3.1.1.1. Acute oral toxicity

Data point	CA 8.3.1.1.1/001
Report author	[redacted]
Report year	2003
Report title	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, *Apis mellifera*
Report No	MON-02-10
Document No	-
Guidelines followed in study	EPPO guideline 170 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations according to guideline OECD 213(1998):* *Minor:* *- Relative humidity was slightly above the recommended range* *- No mortality assessment at 4 hours*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

In a laboratory study, the acute oral toxicity of glyphosate K-salt to the honey bee, *Apis mellifera* L., was established. Following a range finding test, a definitive test was conducted exposing worker bees to nominal doses of 100 µg glyphosate acid equivalent/bee.

Five replicate cages each containing 10 bees (50 bees per control or test group) were prepared for the test item treatment and for the control (50% sucrose only- no test substance). There were three replicates for each of the five reference item treatment groups also prepared. Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation.

At 24 hours, there was a single bee mortality in the control group, with two bee mortalities in the 100 µg a.e./bee test group. At 48 hours, there were a further two bee mortalities in the control with a three additional mortalities in the 100 µg a.e./bee group. The overall control corrected mortality for oral toxicity was 4%. There were no sub-lethal effects observed. All validity criteria according to OECD 213 were fulfilled.

In conclusion, the toxicity of glyphosate K-salt was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was > 104 µg glyphosate acid equivalent/bee.

The study is considered valid so LD_{50} >104 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 78623
Description:	Amber liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	58% K salt of glyphosate, equivalent to 47.3% w/w glyphosate a.e.
Positive control:	Positive control: Dimethoate technical grade
Test organisms:	
Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Roselea Apiaries, East Wellow, Hampshire
Diet/Food:	50 % w/v aqueous sucrose solution
Environmental conditions:	
Temperature:	25 - 26°C
Humidity:	64 – 79%
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	22 July – 27 July 2002

B. STUDY DESIGN

Experimental treatments

A range finding test was conducted using two replicate vessels – each containing 10 bees, at 0.1, 1, 10 and 100 µg a.e//bee and a 50% w/v sucrose control group.

The definitive test was conducted at a single rate (100 µg test item/bee) and included a single control group (50% w/v aqueous sucrose solution).

A toxic reference item (dimethoate) test was conducted in parallel at five test rates (0.200, 0.175, 0.150, 0.125 and 0.100 µg a.s./bee and included a 50% w/v sucrose control group.

Bees were exposed to the test item dispersed in 50% w/v sucrose solution, presented in in narrow glass vials, which were weighed before and after introduction into the three cages per treatment. In the definitive test with MON 78623, at the highest treatment level, the mean dose consumed was 104 µg a.e./bee.

Observations

Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation.

Statistical calculations

Corrected mortality was calculated according to Abbott (1925). LC_{50} values were determined by Probit analysis and the 95% confidence interval by Chi-square goodness of fit test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.3.1.1.1-1: Toxicity of glyphosate K-salt to honey bees (*Apis mellifera* L.) in the oral toxicity test

Dose [µg a.e./bee]	Mean intake of test item [µg a.e./bee]	Mortality [%]			
		1	3	24 h	48 h
Sucrose control	-	0	0	2	6
100	104	0	0	4	10 (4)

In brackets the Abbot corrected mortality is given

B. OBSERVATIONS

No sublethal effects of bees were observed during the 48 hour test period for the test concentration of 104 µg glyphosate acid equivalent/bee and in the sucrose control.

The corrected mortality after 48 h was 4%. The determined 48h LD_{50} for the reference item dimethoate was 0.126 µg/bee for oral toxicity. These results are in line with published values, indicating that the test insects were of suitable sensitivity.

Deviations according to guideline OECD 213(1998):
- Relative humidity was slightly above the recommended range
- No mortality assessment at 4 hours

These deviations are not expected to have a negative impact on the validity of the study.

All validity criteria according to OECD 213 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate acid was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was >104 µg glyphosate acid equivalent/bee.

The study is considered valid so LD_{50} >104 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

This study is valid.

Only a 48h-LD50 was available for the toxic reference in the study report. Based on the effects observed at 24h, it could be estimated that the LD50 would be between 0.13 et 0.16 µg/bee (thus in the range 0.10-0.35 µg dimethoate/bee as recommended in OECD 213). Therefore the validity criterion for the 24h-LD50 is considered fulfilled.

The toxicity of MON 78623 (glyphosate K-salt) was tested in an acute oral toxicity test on honey bees.

oral toxicity test:
LD50 >104 µg glyphosate acid equivalent/bee

Data point	CA 8.3.1.1.1/002
Report author	[redacted]
Report year	1998
Report title	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*)
Report No	FN9700
Document No	-
Guidelines followed in study	EPPO guidelines (1992) OPPTS 850.3020 Draft OECD 213 (1997)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 213 (1998):* *Minor:* *- The starvation of bees before test initiation was 2 h and 10 min, instead of 1-2 h.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
The acute oral toxicity of glyphosate acid to the honey bee *Apis mellifera* L., was determined in a definitive laboratory test with worker bees exposed to nominal doses of 0.0984, 0.984, 9.84, 103 and 206 µg glyphosate acid /bee, presented in 50 w/v sucrose syrup. A reference treatment (dimethoate) group was also included.
Three replicate cages, each containing 10 bees, were prepared for the control and for each test item group and for the reference group. Mortality and sub-lethal effects were assessed 24 and 48 h after test initiation for oral toxicity.
No sub-lethal effects nor mortality of bees was observed after 48 hours of exposure, in the test item and the control groups. All validity criteria according to OECD 213 were fulfilled.
In conclusion, the 48 hour LD_{50} toxicity value for oral exposure of honeybees to glyphosate acid was determined to be >182 µg test item/bee in the oral toxicity test, with a corresponding NOEL of ≥182 µg test item/bee.
The study is considered valid so LD_{50} >182 µg a.s./bee and NOEL of 182 µg a.s./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Technical Glyphosate acid

Description:	White powder
Lot/Batch #:	TSC0521/05148
Purity:	97.6%
2. Vehicle of test material/media and positive control:	Vehicle for positive control: Triton X100 Positive control: Dimethoate (BASF 40 lot 083.10/96)
3. Test organisms:	
Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Own colony
Diet/Food:	Not stated
Environmental conditions:	
Temperature:	25 ± 1°C
Humidity:	65 ± 5%
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	24 August to 04 September 1998

B. STUDY DESIGN

Experimental treatments
The definitive test was conducted with 0.0984, 0.984, 9.84, 103 and 206 µg glyphosate acid/bee, dispersed in 50% w/v aqueous sucrose solution. All test solutions were prepared using an initial stock solution prepared at 103 mg a.s./mL, using deionised water containing 500 mg/L Agral 90. In turn a stock solution at 9.84 mg a.s./mL was prepared and then serially diluted to achieve the required test concentrations. An aliquot of each test concentration (0.5 mL) was diluted to a 10 mL final volume using 50% w/v sucrose solution. The control group received 50% w/v sucrose solution containing 0.5 mL of the 500 mg/L Agral 90.

In the toxic reference group, dimethoate was added to deionised containing 1 g Triton X100/L to achieve 3.5 mg a.s./mL stock solution from which a dilution series was prepared. With a control group of bees receiving 50% w/v sucrose solution containing 0.5 mL Triton X100.

The bees collected from a local hive, were anaesthetised with carbon dioxide immediately before dosing and counted into the mesh covered petri dishes. Each group of 10 bees were offered control, test item or reference item containing feed solutions (0.2 mL) in a glass feeder attached to the mesh cage. The feeders were weighed before and after introduction into the cages. The test was conducted in the dark, with bees held in an incubator at 25 ± 1ºC and 65 ± 5% relative humidity. Duration of uptake was 4 hours for the test item treatments, with all feeders being replaced with fresh feeders containing only 50% sucrose solution.

Observations
Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation.

Statistical calculations
Doses and LD_{50} calculations were based on the analysed content of glyphosate acid. The mortality results were analysed using a probit programme.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.3.1.1.1-2: Toxicity of glyphosate acid to honey bees (*Apis mellifera*) in the oral toxicity test

Dose [µg test item/bee]	Mean intake of glyphosate acid [µg a.s./bee]	Mortality [%]		
		4 h	24 h	48 h
Control	-	0	0	0
0.0984	0.0947	0	0	0
0.984	0.937	0	0	0
9.84	9.7	0	0	0
103	81	0	0	0
206	182	0	0	0

B. OBSERVATIONS

There were no sub-lethal effects nor mortality of bees observed in the 48 hour test period. In the oral toxicity test the maximum nominal test level of 206 µg test item/bee) corresponded to an actual intake of 182 µg a.s./bee.

Deviations according to the current guideline OECD 213:

- The starvation of bees before test initiation was 2 h and 10 min, instead of 1-2 h.

This does not affect the reliability of the study.

All validity criteria according to OECD 213 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate acid was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was > 182 µg a.s./bee, with a corresponding NOEL of ≥182 µg a.s./bee.

The study is considered valid so LD_{50} >182 µg a.s./bee and NOEL of ≥182 µg a.s./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

This study is valid.

The toxicity of glyphosate acid was tested in an oral toxicity test on honey bees.

oral toxicity test:
LD50 (48 h) >182 µg glyphosate acid/bee.

Data point	CA 8.3.1.1.1/003
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Acute contact and oral toxicity to honeybees
Report No	1413/3-1018
Document No	-
Guidelines followed in study	EPPO Guideline No. 170: Test methods for evaluating the side-effects of plant protection products on honeybee (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 213 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours* *- Relative humidity exceeded the recommended values*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
In an acute laboratory study the oral toxicity of glyphosate to honeybee, *Apis mellifera* was tested. After a preliminary dose range-finding test, adult worker bees were treated with 1.25, 2.5, 5.0, 10, 20 and 40 µg glyphosate/bee in the oral test. Three replicate cages, containing 10 bees each, were used. Mortalities and sub-lethal effects were made 1, 4, 24 and 48 h after treatment. No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The validity criteria according to current OECD guideline 213 are fulfilled.
The study is considered valid and the 24 and 48-hour oral LD_{50} values for glyphosate were >40 µg a.s./bee for oral exposure (nominal).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate
Description:	White powder
Lot/Batch #:	H95 D161A
Purity:	95.3%
Vehicle of test material/media and positive control:	Vehicle: reverse-osmosis water Poistive control: formulated Dimethoate (BASF Dimethoate 40 EC)

Test organisms:

Species:	Honey bee (*Apis mellifera*)
Age:	Adult worker bees
Source:	The Bee Farm, Wetherby, West Yorkshire, UK
Diet/Food:	50% sucrose solution *ad libitum*
Acclimatisation:	Not stated

Environmental conditions:

Temperature:	24.5 - 25.8°C

Relative humidity:	49.1 - 86.0%
Photoperiod:	darkness
Experimental dates:	27 June – 06 July 1996

B. STUDY DESIGN

Experimental treatments
To determine the test concentrations for the definitive study a range-finding test was performed. The nominal doses of glyphosate used for the range-finding test were 0, 0.04, 0.4, 4 and 40 µg a.s./bee for oral dosing.
The nominal doses of glyphosate used for the definitive oral test were 0, 1.25, 2.5, 5.0, 10, 20 and 40 µg a.s./bee. Three replicate cages, containing 10 bees each, were used. The reference substance was prepared and dosed in the same media and manner as the test substance doses. The toxic standard test was run in concurrently with the range-finding test and shared the controls. The nominal doses of dimethoate were 0, 0.1, 0.15 and 0.2 µg a.s./bee in the oral test. There were three replicate cages of 10 bees each at each dose level of the reference substance.

Observations
Assessments of mortality and sub-lethal effects were conducted 1, 4, 24 and 48 hours after treatment.

Statistical calculations
Descriptive Statistics; the LD_{50} values of the toxic standard, dimethoate, were calculated by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS
No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The 48 h LD_{50}-value for dimethoate was calculated to be 0.146 µg a.s./bee (95% confidence limits: 0.131 to 0.161) for oral exposure.

Deviations according to the current guideline OECD 213:
- Mortality observation was not assessed at 4 hours
- Relative humidity exceeded the recommended values

These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.

The test is considered to be valid according to OECD guideline 213 as mortality in the negative control did not exceed 10% after 48 hours. In addition, the LD_{50} for the reference item met the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate was tested in an acute oral toxicity test on honey bees. The oral LD_{50} (24 h/48 h) values for glyphosate were >40 µg a.s./bee for oral exposure (nominal).
The study is considered valid so LD_{50} >40 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:
This study is valid.
Only a 48h-LD50 was available for the toxic reference in the study report. Based on the effects observed at 24h, it could be estimated that the LD50 would be around 0.15 µg/bee (thus in the range 0.10-0.35 µg dimethoate/bee as recommended in OECD 213). Therefore the validity criterion for the 24h-LD50 is considered fulfilled.

All oral doses were fully consumed.

The applicant noted that mortality observation was not assessed at 4 hours but this was for the reference toxic not for the test item. Besides no mortality occurred at 48h.
The toxicity of glyphosate was tested in an oral toxicity test on honey bees.

oral toxicity test:
LD50 (48 h) >40 µg a.s./bee nominal.

Data point	CA 8.3.1.1.1/004
Report author	[redacted]
Report year	1995
Report title	Testing Toxicity to Honeybee - *Apis mellifera* L. (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.)
Report No	95 10 48 065
Document No	-
Guidelines followed in study	EPPO Guideline No. 170
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviations from guideline OECD 213 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours.*
Previous evaluation	Yes, accepted in RAR 2015
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
In a laboratory study, the acute oral toxicity of technical glyphosate to the honey bee, *Apis mellifera* L. was tested. Adult worker bees were exposed to two nominal test doses of 100 and 200 µg test item/bee.

In the test, three replicate cages, each containing 10 bees were used for the test item treatment, control and reference treatment. Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after treatment initiation.
Results showed a single bee mortality in the 100 µg a.s./bee treatment group at 24 hours, with no further mortality recorded at 48 hours at both the 100 and 200 µg a.s./bee treatment groups. In addition, no behavioural abnormalities were observed in test item groups and control groups during the whole test period. All validity criteria according to the OECD guideline 213 was fulfilled.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	Not stated
Lot/Batch #:	01/07/95
Purity:	98.2% a.s.
Vehicle of test material/media and positive control:	Dimethoate EC 400, containing 411,14 g a.s./L Extravon (surfactant)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Purchase from the bee-keeper [redacted]
Diet/Food:	50% aqueous sucrose solution *ad libitum* (except for 1 – 2 hours prior to oral test initiation)

Environmental conditions:

Temperature:	25 - 26°C
Humidity:	53 – 70%
Photoperiod:	8 hours diffuse light/16 hours darkness
Experimental dates:	21 August – 01 September 1995

B. STUDY DESIGN

Experimental treatments

The oral toxicity test was conducted with two nominal test doses of 100 and 200 µg a.s./bee. In addition, a control group was fed with 50% sucrose solution. Dimethoate was used a toxic reference, at test doses ranging from 0.20 to 0.40 µg/bee. The oral toxicity test was conducted in triplicate using 10 bees per replicate (30 bees), with the test item or reference item delivered to the bees in 50% sucrose solution in feeding tubes, attached to the bee cages. The bees were fed with 50% aqueous sucrose solutions, containing appropriate concentrations of the test item.

Observations

Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after test start.

Statistical calculations

Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD_{50} value is given below based on nominal concentrations.

Table B.9.3.1.1.1-3: Toxicity of technical glyphosate to honey bees in an oral toxicity tests

Endpoints (48 h)	Technical glyphosate [µg a.s./bee]
Oral LD_{50}	>200

B. OBSERVATIONS

No biologically relevant mortality of bees was observed during the 48-hour test period for test concentrations of up to 200 µg a.s./bee, which was the highest concentration tested. In addition, no behavioural abnormalities were observed at any test item concentration and in the control groups.

For the toxic reference dimethoate, the highest test doses caused 83% and 97% mortalities for oral and contact test respectively.

Table B.9.3.1.1.1-4: Mortality of honey bees in an oral toxicity tests

Test	Time [h]	Mortality [%]			
		Control	Technical glyphosate [µg a.s./bee]		Toxic reference
		-	100	200	Highest test dose (0.4 µg dimethoate EC400/bee)
Oral	24	0	3	0	83
	48	0	3	0	83

Deviations according to the current guideline OECD 213:

- Mortality observation was not assessed at 4 hours.

This deviation is not expected to have a negative impact on the validity of the study.

The validity criteria according to the OECD guideline 213 were fulfilled as the mortality in the control was <10% at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of technical glyphosate was tested in an acute oral toxicity test on honey bees. The LD_{50} (48 h) was >200 µg a.s/bee.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:
This study is valid.

All oral doses were fully consumed.
Toxic reference : LD50 = 0.35 µg dimethoate EC400/bee (thus in the range 0.10-0.35 µg dimethoate/bee as recommended in OECD 213)
The toxicity of glyphosate was tested in an oral toxicity test on honey bees.

oral toxicity test:
LD50 (48 h) >200 µg glyphosate acid/bee (nominal)

Data point	CA 8.3.1.1.1/005
Report author	[redacted]
Report year	1995
Report title	Honey Bees (*Apis mellifera* L.), oral toxicity study in the laboratory with Glyphosate
Report No	141907
Document No	-
Guidelines followed in study	EPPO guidelines 22, 203 – 215 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 213 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours* *- Humidity was lower than the expected range: 34-37% instead of 50-70 %*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

In a laboratory study the acute oral toxicity of glyphosate technical material (96% purity) to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, a definitive test was conducted exposing worker bees to a single nominal dose of 121 µg a.s./bee.

In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and paralysis effects were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment.

No mortality of bees was observed during the 72 hours of exposure. In addition, no paralysis was observed in the test item and the control groups during the 72 hours test period. The validity criteria according to guideline OECD 213 are fulfilled.

In an oral toxicity test, glyphosate had no effects on mortality of honey bees at concentrations of up to and including 116.67 µg a.s./bee (mean (df = 3) actual consumed dose). Therefore, the oral LD_{50} of glyphosate was determined to be >116.67 µg a.s./bee.

The study is considered valid so LD_{50} >116.7 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	GLYFOSAAT (Spelling for report: GLYPHOSATE)
Description:	White powder
Lot/Batch #:	22021
Purity:	96%
Vehicle of test material/media and positive control:	Vehicle: Tap water Positive control: Parathion 25 % liquid

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)

Age: Adult worker bees

Source: Research Centre for Insect Pollination and Beekeeping, "Ambrosiushoeve"

Diet/Food: 50% aqueous sucrose solution *ad libitum* (except during oral dosing and prior starvation)

Environmental conditions:

Temperature: 24 - 25°C

Humidity: 34 – 37%

Photoperiod: 24 hours darkness (except during observation)

Experimental dates: March 08 to March 16 1995

B. STUDY DESIGN

Experimental treatments

Prior to the main test, a range-finding test was performed exposing bees to nominal concentrations of 1.0, 10, 51 and 101 µg a.s./10 µL sucrose solution. The definitive test was conducted as a limit test with a single nominal concentration of 121 µg a.s./10 µL sucrose solution. All test solutions were prepared in a 50 % sucrose solution. In addition, a water-treated control and a reference substance (Parathion 25% liquid) were tested. Food was withheld from the bees for about one to two hours prior to the test. For the test, 10 bees per cage were exposed in triplicate and fed with the test substance suspension. Per group of 10 bees 100 µL test substance suspension was administered (10 µL test solution/bee).

Observations

Mortality, paralysis and any other abnormalities were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment start.

Validity criteria

For a test to be valid the following conditions apply:

- The average mortality for the total number of controls must not exceed 10% at the end of the test.
- The LD_{50} of the toxic standard meets the specified range.

Statistical calculations

Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS

The bees were offered sugar solution containing a concentration of 121 µg a.s./bee. The mean (df = 3) amount of glyphosate consumed by the bees over 72 hours was 116.67 µg a.s./bee. A summary of the mortality is provided below.

Table B.9.3.1.1.1-5: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in an oral toxicity test

Dose [µg a.s./bee]	Intake of test item [µg a.s./bee]	Mortality [%]		
		24 h*	48 h*	72 h*
Control (sugar solution)	-	0.00	3.33	3.33
121	116.67	0	0	0

* Corrected for mortality in the negative control

B. OBSERVATIONS

No mortality of bees was observed at the in the 72 hour limit test at the test concentration of 121 µg a.s./bee. In addition, no paralysis was observed in the test item group and the control group during the 72 hours test period.

Deviations according to the current guideline OECD 213 (1998):

- Mortality observation was not assessed at 4 hours
- Humidity was lower than the expected range: 34-37% instead of 50-70 %

This deviation is not expected to have a negative impact on the validity of the study.

All validity criteria according to OECD 213 were fulfilled, since the average mortality in the control group did not exceed 10% (actual value: 3.33%) and the 24-hour LD_{50} of the toxic standard meets the standard of less than 1.0 µg a.s./bee based on historical data (actual value: 0.4 µg a.s./bee).

In an oral toxicity test, glyphosate had no effects on mortality of honey bees at concentrations of up to and including 116.67 µg a.s./bee.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate was tested in an acute oral toxicity test on honey bees. The LD_{50} (72 h) was >116.67 µg a.s./bee.

The study is considered valid so LD_{50} >116.7 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

This study is valid.

Toxic reference : LD50 = 0.4 µg parathion 25%/bee (no recommended range for parathion in OECD 213), RMS considers that reference toxic performed well.
The toxicity of glyphosate was tested in an oral toxicity test on honey bees.

oral toxicity test:
LD50 (48 h) >116.67 µg glyphosate acid/bee

Data point	CA 8.3.1.1.1/006
Report author	[redacted]
Report year	1972
Report title	The acute contact and oral toxicities of CP67573 and MON2139 to worker honey bees
Report No	HU85X094
Document No	-
Guidelines followed in study	Working Document 13 produced by the UK Pesticide Safety Precautions Scheme
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 213 (1998):* *Major:* *- Mortality in the control was >10% at test termination* *Minor:* *- Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group,* *- Mortality observation was not assessed at 4 hours*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed.
Acceptability/Reliability (RMS)	Invalid

* Two test materials were assessed in this study; namely CP67573 and MON2139 (a 36% w/v formulation). MON2139 contains a surfactant that is not present in the representative formulation for the Annex I renewal. This summary therefore only contains information on CP67573 (glyphosate technical).

Executive Summary

The acute oral toxicity of CP67573 (glyphosate technical) to young adult worker bees (*Apis mellifera* L.) determined in a limit tests performed at a nominal dose of 100 µg a.s./bee. The test comprised 10 replicate mesh cages, each containing 10 bees. In a parallel test, honey bees were exposed to a reference item in a dose response test using dimethoate at concentrations ranging from 0.048 to 0.117 µg dimethoate/bee. In both tests, the test substance was suspended in 20% sucrose and 0.2 mL was fed to each replicate of 10 bees. Control groups consisting of 2 cages of 10 bees were included alongside each of the tests.

Assessments of mortality were conducted after 24 and 48 hours. The validity criteria according to OECD guideline 213 were not fulfilled as mortality in the control was >10% at test termination.

In the 100 µg CP67573/bee treatment group, at 24 and 48 hours, there was 46% and 56% mortality, with corresponding mortality in the control group of 10% and 15%, respectively.

This resulted in overall control corrected mortality levels of 40 and 48% achieving a 48 hour LD_{50} of 100 µg a.s./bee. The study is considered invalid as mortality in the control was >10% at test termination.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: CP67573 (technical active ingredient)

Description: Not stated

Lot/Batch #: No batch details presented in report

Purity: Not stated

Density: Not stated

Positive control:	Positive control: Dimethoate
Test organisms:	
Species:	Honey bee (*Apis mellifera*)
Age:	Young adult worker bees
Source:	Experienced apiarist in Huntingdonshire, U.K
Diet/Food:	Bees were fed with 20% sucrose
Acclimatisation:	Not reported
Environmental conditions:	
Temperature:	26 – 27°C
Relative humidity:	Not reported
Photoperiod:	Not reported
Experimental dates:	Not reported

B. STUDY DESIGN

Experimental treatments
Honey bees were exposed orally to CP67573 in a limit test conducted at 100 µg a.s./bee, in nylon coated 2 mm wire mesh tubes, with 11.5 cm high and 3.5 cm in diameter, closed by corks at both ends. Bees were placed in each cage and were fed with 20 % sucrose. For the oral toxicity tests, compounds were suspended in 20% sucrose and 0.2 mL was fed to each replicate of 10 bees.
There were 10 cages per test item treatment, with two control cages containing 10 worker bees each. A reference item dose-response test (dimethoate) was conducted in parallel, at five test rates between 0.048 and 0.117 µg test item/bee, with two cages of ten bees per treatment and control group.

Mortality in the test or reference item treatment groups, were corrected for control mortalities using Abbot's correction, to give overall control corrected levels of mortality, on which the endpoint LD_{50} values were based.

Observations
Mortality was recorded 24 and 72 hours after test initiation.

Statistical calculations
Descriptive statistics; LD_{50} for dimethoate were obtained by graphical interpolation on probability/log paper, confidence limits were calculated according to Litchfield & Wilson (1949).

II. RESULTS AND DISCUSSION

A. FINDINGS
A summary of the mortality results is provided below.

Table B.9.3.1.1.1-6: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in an oral toxicity test

Endpoints (48 h)	CP67573 [µg a.s./bee]
LD_{50} oral	100

Table B.9.3.1.1.1-7: Oral toxicity of CP67573 to honey bees (*Apis mellifera* L.)

Exposure	Mortality [%]		Corrected mortality [%]
	Control	100 µg/bee	
oral (24 h)	10	46	40
oral (48 h)	15	56	48

B. OBSERVATIONS

In the test with CP67573, the corrected bee mortality did not reach or exceed 50% (max mortality was 48%), resulting in overall control corrected mortality levels of 40 and 48% at 24 and 48 hour respectively, achieving a 48 hour LD_{50} of 100 µg a.s./bee.

In the reference item test with dimethoate, a 48 hour oral exposure LD_{50} value of 0.056 µg dimethoate/bee (95% C.I. of 0.045 - 0.070 µg dimethoate/bee) was observed.

Deviations according to the current guideline OECD 213:

- Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group,
- Mortality observation was not assessed at 4 hours

These deviations are not expected to have a negative impact on the validity of the study.

- Mortality in the control was >10% at test termination.

This deviation has a negative impact on the validity of the study.

The validity criteria according to the OECD guidelines 213 were not fulfilled as mortality in the control was >10% at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of CP67573 was tested in an acute oral toxicity test on honey bees. The oral LD_{50} (48 h) were 100 µg a.s./bee.

The study is considered invalid as mortality in the control was >10% at test termination.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).

This study was not conducted under GLP (GLP was not compulsory at the time the study was performed).

RMS notes that 20% sucrose was fed to bees (instead of 50% according to OECD 213). However, RMS agrees with the applicant, that control mortality exceeded the validity criteria.

This study is not valid.

Data point	CA 8.3.1.1.1/007
Report author	[redacted]
Report year	2017
Report title	MON 0139: Acute Oral and Contact Toxicity to the Bumble Bee, *Bombus terrestris* L. under Laboratory Conditions
Report No	S16-06634
Document No	-
Guidelines followed in study	Based on the proposal for new OECD Guidelines: Bumblebee, acute oral toxicity test (2016) and Bumblebee, acute contact toxicity test (2016)
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviation from OECD guideline 247 (2017): none*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability **(RMS)**	Valid

Executive Summary

The acute oral toxicity of MON 0139 to bumblebees (*Bombus terrestris*) was established in a 48 hour laboratory toxicity test, with bees exposed at five test rates (62.5, 125, 250, 500 and 1000 µg product/bumble bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./bumble bee, via oral ingestion in aqueous sucrose solution. In the main test, for the control and test group, there were 35 individually housed bumblebees, with application solutions (50% w/v sucrose solution) presented in plastic feeder syringes.

A reference item test was conducted in parallel with bumble bees exposed to dimethoate at 1.5 µg a.s./bumble bee, with exposure of 32 individually housed bumblebees via 50% w/v sucrose solution in syringe feeders.

Mortality assessments were made at 4, 24 and 48 hours after application (after start of feeding in the oral toxicity test). Observations for sublethal effects were recorded at each observation interval.

There was 100% mortality in the reference item test demonstrating the test system as being appropriate and the bumblebees were sensitive.

In the main study, the 48 hours oral LD_{50} (Lethal Dose causing 50% mortality) for MON 0139 was determined to be >894 µg product/bumble bee (equivalent to >412 µg a.e./bumble bee). The NOED for mortality after 48 hours was determined to be 894 µg product/bumble bee (equivalent to 412 µg a.e./bumble bee).

The validity criteria for the control group in the main test and reference item mortality were met and thus, the test was considered valid.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Test item:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (a.e.); 574.4 g/ml
Description:	liquid / slightly yellow
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.2460 g/cm^3

Treatments

Test rates:	Oral toxicity test: Target doses: 62.5, 125, 250, 500 and 1000 µg prod./bumble bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./bumble bee Actual uptake: 56.9, 113, 226, 453 and 894 µg prod./bumble bee, equivalent to 26.2, 52.1, 104, 209 and 412 µg a.e./bumble bee
Control:	Pure 50% (w/v) aqueous sucrose solution
Toxic standard:	BAS 152 11 I (dimethoate, analysed 405.2 g a.s./L) 1.5 µg a.s./bumble bee (target doses) 1.36 µg a.s./bumble bee (actual uptake)
Administration:	Oral: ingestion in 50% w/v aqueous sucrose solution.

Test organisms

Species:	*Bombus terrestris* L. (Hymenoptera: Apidae)
Source:	From healthy colony owned and maintained by Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium.
Food:	50% w/v aqueous sucrose solution

Test design

Test cage description:	Nicot cages with plastic syringe feeders attached.
Replication:	35
No. of bees/arena:	1
Duration of test:	48 hours

Environmental conditions

Temperature:	24.8 – 25.3°C
Humidity:	50.9 ± 60.4%
Photoperiod:	Darkness (except during application and observations)
Experimental dates:	10 April to 13 April 2017

B. STUDY DESIGN

Experimental treatments

Adult worker bumblebees (*Bombus terrestris*) were exposed to MON 0139 via oral ingestion in aqueous sucrose solution. To immobilise the bumblebees during the course of treatment, they were anaesthetised using CO_2. Bumblebees were starved for 2 hours until treatment, to ensure that the bees were equal in terms of their gut contents at the start of the test. Each bumblebee was offered 40 µL of the test material or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in this 40 µL. The doses were measured into the feeding tubes and the weights of

these were recorded before the doses were made available to the bumblebees. After four hours, the feeding tubes were replaced with similar tubes containing untreated 50% w/v aqueous sucrose solution supplied *ad libitum*. All feeding tubes with test solutions were weighed in order to calculate actual mean consumption per bee for each treatment.

Assessments
Mortality was recorded 4 and 24 hours after application (after start of feeding in the oral toxicity test) and thereafter at 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. In the reference item group, behavioural assessments were not conducted as it was assumed that moribund and affected bumble bees of the reference item group would die by the end of the test.

Statistics
For the statistical evaluation the statistics program ToxRat professional, Version 3.2.1 was used. Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment groups in the oral toxicity test and to determine the NOED based on mortality.

The LD_{50} with 95% confidence limits could not be calculated in the oral toxicity test since the observed mortalities were below 50% in all test item groups. Statistical evaluation was not necessary in the oral toxicity test, since no mortality occurred in any test item treatment group or the control group.

II. RESULTS AND DISCUSSION

A. FINDINGS

In the control group fed with pure 50% (w/v) aqueous sucrose solution, no mortality was observed at the final assessment after 48 hours. In the test item treatment group, no mortality was observed at any target dose 48 hours after start of feeding. No treatment related behavioural abnormalities were recorded during the 48 hour testing period at any target dose.

Table B.9.3.1.1.1-8: Summary of oral acute toxicity of MON 0139 to the bumblebee

MON 0139	**Oral toxicity test**	
	[µg product/bumble bee]	**[µg a.e./bumble bee]**
LD_{50} (24 h)	>894	>412
LD_{50} (48 h)	>894	>412
NOED (48 h)	≥894	≥412

Validity criteria
The study is considered valid since the control and reference item validity criteria were met:
The mean control mortality was ≤10% at the end of the test;
The mean reference item mortality was ≥50% at the end of the test

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 hours oral LD_{50} for MON 0139 was determined to be >894 µg product/bumble bee, equivalent to >412 µg a.e./bumble bee. The NOED for mortality after 48 hours was determined to be ≥894 µg product/bumble bee, equivalent to ≥412 µg a.e./bumble bee.
The study is considered valid.
The LD_{50} >412 µg glyphosate acid/bumble bee and NOED ≥412 µg glyphosate acid./bumble bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:
New study.
Test item: MON 0139 containing glyphosate IPA salt (46.1% (w/w).

RMS notes that analytical verification of dose is missing (this was not a requirement at the time of study conduct). However, this is not part of the validity criteria of the OECD 247 guideline. Moreover, analytical verification is not a requirement for other acute tests on bees (OECD 213, 214). Thus RMS proposed to consider the results as reliable for risk assessment.

This study is valid.
48h Oral – LD50 > 894 µg MON 0139/bumble bee (equivalent 412 µg glyphosate./L)

B.9.3.1.1.2. Acute contact toxicity

Data point:	CA 8.3.1.1.2/001
Report author	[redacted]
Report year	2003
Report title	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, *Apis mellifera*
Report No	MON-02-10
Document No	-
Guidelines followed in study	EPPO guideline 170 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 214 (1998):* *Minor:* *- Relative humidity was slightly above the recommended range* *- No mortality assessed at 4 hours.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary
In a laboratory study the acute contact toxicity of glyphosate K-salt to the honey bee, *Apis mellifera* L., were tested. Following a range finding test, a definitive test was conducted exposing worker bees to nominal doses of 100 µg glyphosate acid equivalent/bee.
Five replicate cages, each containing 10 bees, were used for the test item treatments, controls and three for the reference treatments. Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation. Corrected mortality for contact toxicity was 0%. No sublethal effects were observed except for one bee one hour after test item application. All validity criteria according to OECD 214 were fulfilled.
In conclusion, the toxicity of glyphosate K-salt was tested in an acute contact toxicity test on honey bees.
The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 78623
Description:	Amber liquid
Lot/Batch #:	GLP-0108-11688-F
Purity:	58% K salt of glyphosate, equivalent to 47.3% w/w glyphosate a.e.
Vehicle of test material and positive control:	Vehicle for test item: Farmon Blue (87.3% w/w alkyl phenol ethylene oxide) / Positive control: Dimethoate technical grade

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Roselea Apiaries, East Wellow, Hampshire
Diet/Food:	50% w/v aqueous sucrose solution

Environmental conditions:

Temperature:	25 - 26°C
Humidity:	64 – 79%
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	22 July – 27 July 2002

B. STUDY DESIGN

Experimental treatments

Following an initial range-finding test, the definitive test was conducted as a limit test with 100 µg glyphosate acid equivalent/bee, prepared in an appropriate carrier (0.05% solution of the wetting agent Farmon Blue) and administered as a 1.0 µL droplet per bee (dorsal thorax) to each of ten bees in each of five cages per treatment. A vehicle control containing 0.05 w/v solution of Farmon Blue and deionised water and a toxic reference solution containing dimethoate were run in parallel. During the observation method a 50% w/v aqueous sucrose solution was provided.

Observations

Mortality and sub-lethal effects were assessed 1, 3, 24 and 48 h after test initiation.

Statistical calculations

Corrected mortality was calculated according to Abbott (1925). LC_{50} values were determined by Probit analysis and the 95% confidence interval by Chi-square goodness of fit test.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.3.1.1.2-1: Toxicity of glyphosate K-salt to honey bees (*Apis mellifera* L.) in the contact toxicity test

Dose [µg a.e./bee]	Mean intake of test item [µg a.e./bee]	Mortality [%]			
		1	3	24 h	48 h
Contact toxicity test					
Control	-	0	0	2	4
Farmon Blue control	-	0	0	2	4
100	-	0	0	2	2 (0)

In brackets the Abbot corrected mortality is given

B. OBSERVATIONS

Corrected mortality at 48 h was 0%. No sublethal effects were observed except for one bee one hour after test start, but it recovered by 3 h.
The determined contact 48h LD_{50} for the reference item dimethoate was 0.123 µg/bee for contact toxicity. These results are in line with published values, indicating that the test insects were of suitable sensitivity.

Deviations according to the current guideline OECD 214:

- Relative humidity was slightly above the recommended range
- No mortality assessed at 4 hours.

These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.

All validity criteria according to OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >100 µg glyphosate acid equivalent/bee in the contact toxicity test.

The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:
This study is valid.

Only a 48h-LD50 was available for the toxic reference in the study report. Based on the effects observed at 24h, it could be estimated that the LD50 would be between 0.1 and 0.125 µg/bee (thus in the range 0.10-0.30 µg dimethoate/bee as recommended in OECD 213). Therefore the validity criterion for the 24h-LD50 is considered fulfilled.

The toxicity of MON 78623 (glyphosate K-salt) was tested in an acute contact toxicity test on honey bees.

contact toxicity test:
LD50 >100 µg glyphosate acid equivalent/bee

Data point	CA 8.3.1.1.2/002
Report author	[redacted]
Report year	2000
Report title	Acute Contact Toxicity of GLIFOSATO IPA TECHNICO [redacted] to Honey Bee (*Apis mellifera* L.)
Report No	RF-D4.017/00
Document No	-
Guidelines followed in study	OECD Draft Proposal for a New Guideline: Honey bees, Acute Contact Toxicity Test (1996).
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 214 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours* *- A water control and an undosed control were reported in chapter 5.7.4 (Experimental test), however results of only one (negative) control group were reported.* *- The temperature in test cages was higher than the expected range: 27-31°C instead of 25±2°C.* *- Humidity was lower than the expected range: 39-67% instead of 50-70%* *- 24-hour LD_{50} with dimethoate is slightly above the requested range of 0.10-0.30 µg a.s./bee.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

In an acute laboratory study the contact toxicity of isopropylamine (IPA) salt of glyphosate to the honey bee, *Apis mellifera* L. was tested. Following a range finding test, adult worker bees were exposed to nominal dose rates of 10.0, 12.5, 24.0, 62.5 and 100.0 µg glyphosate IPA salt/bee. In addition, an untreated control was tested. Technical dimethoate was used as a reference item.

In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and sublethal effects were recorded at 24 and 48 hours after the treatment.

No significant mortality of bees was observed during the 48 hours observation period. In addition, no sublethal effects were observed. The validity criteria according to guideline OECD 214 are fulfilled.

In conclusion, under the conditions of the present test, the 48 hours contact LD_{50} of was determined to be >100 µg glyphosate IPA salt/bee, equivalent to >61.3 µg a.e./bee.

This study is considered valid in spite of slightly higher LD_{50} for the reference toxicant so LD_{50} >61.3 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate isopropylamine salt (technical)
Description:	Not stated
Lot/Batch #:	MJRT 02 S 201 04
Purity:	612.7 g/kg salt equivalent (analysed)
Density:	Not stated
Vehicle of test material and/or positive control:	Vehicle: water + acetone Positive control: technical dimethoate

Test organisms:

Species:	Honey bee (*Apis mellifera*)
Age:	Adult worker bees from healthy colonies
Source:	Apiario Silva Unit, Piracicaba, Brasil
Diet/Food:	Sucrose solution *ad libitum*
Acclimatisation:	At 25 ± 2 °C and 65 ± 5% relative humidity between collection of worker bees and test initiation (time span not stated)

Environmental conditions:

Temperature:	27 – 31°C
Relative humidity:	39 – 67%
Photoperiod:	24 hours darkness
Experimental dates	05 June – 14 June 2000

B. STUDY DESIGN

Experimental treatments
Based on the results of a range-finder test, bees in the main test were exposed to the nominal dose rates of 10.0, 12.5, 24.0, 62.5 and 100.0 µg glyphosate IPA salt/bee. The glyphosate concentration was analysed in each of the dosing solutions. In addition, an undosed control was tested. Technical dimethoate was used as a reference item. The test was conducted with 3 replicates chambers (inverted petri dish (50 mm depth x 100 mm diameter) per test concentration/control and 10 bees per cage. Bees were anaesthetised with carbon dioxide and counted onto filters papers inside each petri dish in groups of 5 until all chambers contained 10 bees. Bees were exposed to either the test material, the reference toxicant, water or acetone, by administering 1.0 □L of the appropriate substance to the ventral side of the thorax, using a micro syringe. After dosing the cages, a smaller inverted petri-dish containing sucrose solution was placed inside each chamber, and the chambers were covered with a 100-gauge mesh tissue 'lid' to prevent bee escape. All chambers were kept in darkness for 48 hours. Sucrose solution was available *ad libitum* throughout the whole test period.

Observations
Mortality and sublethal effects were recorded at 24 and 48 hours after treatment.

Validity criteria
For a test to be valid the following conditions apply:

- the average mortality for the total number of controls must not exceed 10% at the end of the test;
- the LD_{50} of the toxic standard meets the specified range.

Statistical calculations
Descriptive statistics for the test item. Data on mortality for dimethoate were analysed using Trimmed Spearman-Karber Method.

II. RESULTS AND DISCUSSION

A. FINDINGS

The measured test concentrations ranged between 90.35 and 103.5% of the nominal values.

Table B.9.3.1.1.2-2: Analytical results

Nominal concentration (g glyphosate IPA salt/L)	Measured concentration (g/L)	Concentration expressed as % of nominal (%)	% of deviation from the nominal
Control	-	-	-
10	10.350	103.50	3.50
12.5	12.778	102.22	2.22
24	23.722	98.84	1.16
62.5	60.369	96.59	3.41
100	90.350	90.35	9.65

Analytical data: Analytical determination of the test concentrations showed that the deviation from the nominal concentrations was below 20%. Therefore, the ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

A summary of the mortality is provided below.

Table B.9.3.1.1.2-3: Toxicity of glyphosate IPA salt to honey bees (*Apis mellifera*) in a contact toxicity test

Dose [µg glyphosate IPA salt/bee]	Mortality (mean of 3 replicates) [%]	
	24 h	48 h
Control (undosed)	0.0	0.0
10.0	0.0	0.0
12.5	0.0	0.0
24.0	0.0	0.0
62.5	0.0	0.0
100.0	3.33	3.33

Reference test: The determined 24 h LD_{50} for the reference item was 0.34 µg dimethoate/bee and 48 h LD_{50} for the reference item was 0.12 µg dimethoate/bee. These results show a toxicity level just above the ranges reported by the OECD guidelines.

B. OBSERVATIONS

No sub-lethal effects were observed up to a dose of 100 µg glyphosate IPA salt/bee, equivalent to 61.3 µg a.e./bee. The highest dose that showed no lethal effect was 62.5 µg glyphosate IPA salt/bee.

The test is considered to be valid because the negative control mortality did not exceed 10% (actual value: 0%) and the 24-hour LD_{50} of the toxic standard was slightly above the range of 0.10-0.30 µg a.s./bee specified in the guideline 214 (actual value: 0.34 µg dimethoate/bee).

The following points are deviated from the current guideline but are not expected to have any negative on the study validity:

- Mortality observation was not assessed at 4 hours
- A water control and an undosed control were reported in chapter 5.7.4 (Experimental test), however results of only one (negative) control group were reported.
- The temperature in test cages was higher than the expected range: 27-31°C instead of 25 ± 2°C.
- Humidity was lower than the expected range: 40 - 67% instead of 50 - 70%

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate IPA salt was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >100 µg glyphosate IPA salt/bee, equivalent to >61.3 µg a.e./bee.

This study is considered valid in spite of slightly higher LD_{50} for the reference toxicant so o LD_{50} >61.3 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

The LD50 for the toxic reference was slightly above the required range. RMS agrees with the applicant that it has no significant impact on the outcome of the study.

This study is valid.
The toxicity of glyphosate IPA salt was tested in an acute contact toxicity test on honey bees.
contact toxicity test:
LD50 >100 µg glyphosate IPA salt /bee (technical) equivalent to >61.3 µg /bee (salt equivalent)

Data point:	CA 8.3.1.1.2/003
Report author	[redacted]
Report year	1998
Report title	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*)
Report No	FN9700
Document No	-
Guidelines followed in study	EPPO guidelines (1992) OPPTS 850.3020 Draft OECD 213 (1997) and Draft OECD 214 (1997)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 214 (1998): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
In an acute laboratory study the contact toxicity of glyphosate acid to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, a definitive test was conducted exposing female worker bees to nominal doses of 0.0984, 0.984, 9.84 and 103 µg glyphosate acid/bee.
Three replicate cages, each containing 10 bees, were used for the test item treatments, controls and reference treatments. Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for contact toxicity.
No mortality of bees or sub-lethal effects were observed after 48 hours of exposure in the test item and the control groups during the 48 hours test period. All validity criteria according to OECD 214 were fulfilled as mortality in the control group did not exceed 10% (actual 0%) and the LD_{50} of the toxic standard met the specified range.
In conclusion, the toxicity of glyphosate acid was tested in an acute contact and an oral toxicity test on honey bees.
The study is considered valid so LD_{50} >103 µg a.s./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Technical Glyphosate acid
Description:	White powder
Lot/Batch #:	TSC0521/05148
Purity:	97.6%
Vehicle of test material and/or positive control:	Vehicle for test item: Agral 90 Vehicle for positive control: Triton X100 Positive control: Dimethoate (BASF 40 lot 083.10/96)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Own colony
Diet/Food:	Not stated
Environmental conditions:	
Temperature:	25 ± 1°C
Humidity:	65 ± 5%
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	24 August - 04 September 1998

B. STUDY DESIGN

The definitive test was conducted with 0.0984, 0.984, 9.84 and 103 µg glyphosate acid/bee prepared in an appropriate carrier (deionised water containing 500 mg/L of the wetting agent Agral 90) and administered as a 1.0 µL droplet per bee (dorsal thorax) to each of ten bees in each of three cages per treatment.. A control with 500 mg Agral 90/L and a toxic reference solution containing 1g Triton X100/L were run in parallel. During the observation method a 50% w/v aqueous sucrose solution was provided.

Observations
Mortality and sub-lethal effects were assessed 4, 24 and 48 h after test initiation for contact toxicity.

Statistical calculations
Doses and LD_{50} calculations were based on the analysed content of glyphosate acid. The mortality results were analysed using a probit programme (toxic reference treatment).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.3.1.1.2-4: Toxicity of glyphosate acid to honey bees (*Apis mellifera*) in the contact toxicity test

Dose [µg test item/bee]	Mean intake of glyphosate acid [µg a.s./bee]	Mortality [%]		
		4 h	24 h	48 h
Contact toxicity test				
Control	-	0	0	0
0.0984	-	0	0	0
0.984	-	0	0	0
9.84	-	0	0	0
103	-	0	0	0

B. OBSERVATIONS

No mortality of bees was observed in the 48 hours test period. No sub-lethal effects were observed in the test item group and the control group during the 48 hours test period.

All validity criteria according to OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10% and the LD_{50} of the toxic standard meets the specified range.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate acid was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >103 µg glyphosate acid/bee.
The study is considered valid so LD_{50} >103 µg a.s./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:
This study is valid.

The toxicity of glyphosate acid was tested in a contact toxicity test on honey bees.

contact toxicity test:
LD50 (48 h) >103 µg glyphosate acid/bee.

Data point	CA 8.3.1.1.2/004
Report author	[redacted]
Report year	1996
Report title	Glyphosate: Acute contact and oral toxicity to honeybees.
Report No	1413/3-1018
Document No	-
Guidelines followed in study	EPPO Guideline No. 170: Test methods for evaluating the side-effects of plant protection products on honeybee (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 214 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours* *- The relative humidity exceeded the recommended values*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive summary
In an acute laboratory study the contact toxicity of glyphosate to honeybee, *Apis mellifera* was tested. After a preliminary dose range-finding test, adult worker bees were treated with 0, 0.625, 1.25, 2.5, 5.0, 10 and 20 µg glyphosate/bee in the contact test. Three replicate cages, containing 10 bees each, were used. Mortalities and sub-lethal effects were made 1, 4, 24 and 48 h after treatment. No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The validity criteria according to current OECD guideline 214 are fulfilled.

In conclusion the 24 and 48-hour oral LD_{50} values for glyphosate were >20 µg a.s./bee for contact exposure (nominal).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: Glyphosate
Description: White powder

Lot/Batch #:	H95 D161A
Purity:	95.3%
Vehicle of test material and/or positive control:	Vehicle: Headland Enhance LF + reverse-osmosis water Positive control: formulated Dimethoate (BASF Dimethoate 40 EC)
Test organisms:	
Species:	Honey bee (*Apis mellifera*)
Age:	Adult worker bees
Source:	The Bee Farm, Wetherby, West Yorkshire, UK
Diet/Food:	50% sucrose solution *ad libitum*
Acclimatisation:	Not stated
Environmental conditions:	
Temperature:	24.5 - 25.8°C
Relative humidity:	49.1 - 86.0%
Photoperiod:	darkness
Experimental dates:	27 June – 06 July 1996

B. STUDY DESIGN

Experimental treatments
To determine the test concentrations for the definitive study a range-finding test was performed. The nominal doses of glyphosate used for the range-finding test were 0, 0.1, 1, 10 and 20 µg a.s./bee for contact dosing.
Bees were anaesthetised with carbon dioxide. Contact doses were applied as a 1.0 µL droplet of the test solution was placed on the dorsal thorax of each bee. The nominal doses of glyphosate used for the definitive test contact were 0, 0.625, 1.25, 2.5, 5.0, 10 and 20 µg a.s./bee. The nominal dose of 20 µg a.s./bee was given as a double droplet application (2 × 1 µL). Three replicate cages, containing 10 bees each, were used.

Observations
Assessments of mortality and sub-lethal effects were conducted 1, 4, 24 and 48 h after treatment.

Statistical calculations
Descriptive Statistics; the LD_{50} values of the toxic standard, dimethoate, were calculated by Probit analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortalities or sub-lethal effects were seen in any treatment or controls over the 48 h definitive test period. The 48 h LD_{50}-value for dimethoate was calculated to be 0.452 µg a.s./bee (95% confidence limits: 0.374 to 0.557) for contact exposure.

Deviations according to the current guideline OECD 214:
- Mortality observation was not assessed at 4 hours
- The relative humidity exceeded the recommended values

These deviations are not expected to have a negative impact on the validity of the study which was valid at the time of conduct.

The test is considered to be valid according to OECD guideline 214 as mortality in the negative control did not exceed 10% after 48 hours. In addition, the LD_{50} for the reference item met the specified range.

II. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of glyphosate was tested in an acute contact toxicity test on honey bees. The contact LD_{50} (24 h/48 h) values for glyphosate were >20 µg a.s./bee for contact exposure (nominal).
The study is considered valid so LD_{50} >20 µg a.e./bee can be for risk assessment purposes.

Assessment and conclusion by RMS:
This study is valid.
The applicant noted that mortality observation was not assessed at 4 hours but this was for the reference toxic not for the test item. Besides no mortality occurred at 48h.
Only a 48h-LD50 was available for the toxic reference in the study report. Based on the effects observed at 24h, it could be estimated that the LD50 would be around 0.4 µg/bee (thus slightly above the range 0.10-0.30 µg dimethoate/bee as recommended in OECD 214). RMS considers this deviation as minor and that it has no significant impact on the outcome of the study.
The toxicity of glyphosate was tested in a contact toxicity test on honey bees.

contact toxicity test:
LD50 (48 h) >20 µg glyphosate acid/bee

Data point	CA 8.3.1.1.2/005
Report author	[redacted]
Report year	1995
Report title	Testing Toxicity to Honeybee - *Apis mellifera* L. (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.)
Report No	95 10 48 065
Document No	-
Guidelines followed in study	EPPO Guideline No. 170
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 214 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
In a laboratory study, the acute contact toxicity of technical glyphosate to the honey bee, *Apis mellifera* L. was tested. Adult worker bees were exposed to two nominal test doses of 100 and 200 µg a.s/bee. In the test, three replicate cages, each containing 10 bees were used for the test item treatment, control

and reference treatment. Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after treatment initiation.
In the contact exposure test, there was no bee mortality recorded during the 48 hours test period at both test rates. In addition, no behavioural abnormalities were observed in test item groups and control groups during the whole test period. All validity criteria according to the OECD guideline 214 was fulfilled.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical
Description:	Not stated
Lot/Batch #:	01/07/95
Purity:	98.2% a.s.
Vehicle of test material and/or positive control:	Positive control: Dimethoate EC 400, containing 411.14 g a.s./L Vehicle: Extravon (surfactant)

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Adult worker bees
Source:	Purchase from the bee-keeper [redacted]
Diet/Food:	50% aqueous sucrose solution *ad libitum* (except for 1 – 2 hours prior to oral test initiation)

Environmental conditions:

Temperature:	25 - 26°C
Humidity:	53 - 70%
Photoperiod:	8 hours diffuse light/16 hours darkness
Experimental dates:	21 August – 01 September 1995

B. STUDY DESIGN

Experimental treatments

The contact toxicity test was performed at two nominal test doses of 100 and 200 µg a.s./bee, with the test substance dissolved into a 1 % watery solution/surfactant Extravon. A negative control group where bees were exposed to 0.1 % Extravon only was also included. Dimethoate was used a toxic reference, at test doses ranging from 0.0313 to 1.0 µg/bee. The contact toxicity test was conducted in triplicate using 10 bees per replicate (30 bees). For contact toxicity test, test solutions containing appropriate concentrations of technical glyphosate were dosed to bees by thorax injection. After administration of the test substance, the bees were provided with 50% sucrose solution.

Observations

Mortality, poisoning symptoms and behavioural abnormalities were recorded 24 and 48 hours after test start.

Statistical calculations

Descriptive statistics

II. RESULTS AND DISCUSSION

A. FINDINGS

The LD_{50} value is given below based on nominal concentrations.

Table B.9.3.1-2: Toxicity of technical glyphosate to honey bees in a contact toxicity tests

Endpoints (48 h)	Technical glyphosate [µg a.s./bee]
Contact LD_{50}	>200

B. OBSERVATIONS

No biologically relevant mortality of bees was observed during the 48-hour test period for test concentrations of up to 200 µg a.s./bee, which was the highest concentration tested. In addition, no behavioural abnormalities were observed at any test item concentration and in the control groups.

For the toxic reference dimethoate, the highest test doses caused 97% mortalities for contact test.

Table B.9.3.1-3: Mortality of honey bees in a contact toxicity tests

Test	Time [h]	Mortality [%]			
		Control	Technical glyphosate [µg a.s./bee]		Toxic reference [µg a.s./bee]
		-	100	200	Highest test dose (1 µg dimethoate EC400/bee)
Contact	24	0	3	3	97
	48	0	0	0	97

Deviations according to the current guideline OECD 214:

- Mortality observation was not assessed at 4 hours.

This deviation is not expected to have a negative impact on the validity of the study.

The validity criteria according to the OECD guideline 214 was fulfilled as the mortality in the control was <10% at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The toxicity of technical glyphosate was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >200 µg a.s/bee.
The study is considered valid so LD_{50} >200 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:
This study is valid.

Toxic reference : LD50 = 0.41 µg dimethoate EC400/bee (thus in the range 0.10-0.30 µg dimethoate./bee as recommended in OECD 214, validity criteria fulfilled)

The toxicity of glyphosate was tested in a contact toxicity test on honey bees.

contact toxicity test:
LD50 (48 h) >200 µg a.s./bee

Data point:	CA 8.3.1.1.2/006
Report author	[redacted]
Report year	1995
Report title	Honey Bees (*Apis mellifera* L.), contact toxicity study in the laboratory with Glyphosate
Report No	142335
Document No	-
Guidelines followed in study	EPPO guidelines 22, 203 – 215 (1992)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 214 (1998):* *Minor:* *- Mortality observation was not assessed at 4 hours* *- Humidity was lower than the expected range: 34-40% instead of 50-70%* *- Test extended to 72h with no rising of mortality of 10 %. Additional assessment in regards to guideline requirement.* *- Water control was not setup.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
In an acute laboratory study the contact toxicity of glyphosate technical material (96 % purity) to the honey bee, *Apis mellifera* L., was tested. Following a range finding test, adult worker bees were exposed to a single nominal dose of 100 µg a.s./bee.
In the test, three replicate cages, each containing 10 bees, were used for the test item treatment, control and reference treatment. Mortality and paralysis effects were recorded at least at the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment.
No mortality of bees was observed after 72 hours of exposure. In addition, no paralysis was observed in the test item and the control groups during the 72 hours test period. The validity criteria according to guideline OECD 214 are fulfilled.
In a contact toxicity test, no effects of glyphosate on the mortality and the paralysis of honey bees were observed at concentrations up to and including 100 µg a.s./bee.
The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	GLYFOSAAT (Spelling for report: GLYPHOSATE)
Description:	White powder
Lot/Batch #:	22021
Purity:	96%

Vehicle of test material and positive control: Vehicle of test material/media: Tap water + acetone
Positive control: Parathion 25 % liquid

Test organisms:

Species:	Honey bee (*Apis mellifera* L.)
Age:	Naïve worker bees
Source:	Research Centre for Insect Pollination and Beekeeping, "Ambrosiushoeve"
Diet/Food:	50% aqueous sucrose solution *ad libitum* (except during treatment)
Environmental conditions:	
Temperature:	24 - 25°C
Humidity:	34 – 40%
Photoperiod:	24 hours darkness (except during observation)
Experimental dates:	20 March - 25 March 1995

B. STUDY DESIGN

Experimental treatments
Prior to the main test, a range-finding test was performed exposing adult bees to nominal concentrations of 1.0, 10, 50 and 99 µg a.s./1 µL acetone. The definitive test was conducted as a limit test with a single nominal concentration of 100 µg a.s./1 µL acetone. All test solutions were prepared in an acetone solution. In addition, a control constituted of acetone and the reference substance (Parathion 25% liquid) were tested.
For the definite test, adult worker bees were exposed in triplicates (10 bees/test cage) to the test item, control and reference item. After the test substance was applied on the ventral part of the thorax of the bees with a micropipette ($1mm^3$/bee), then the bees were provided with sucrose solution 50%.

Observations
Mortality, paralysis and any other abnormalities were recorded at least the following approximate time intervals: 30, 60, 90 and 120 minutes after treatment and 24, 48 and 72 hours after treatment start.

Validity criteria
For a test to be valid the following conditions apply:

- the average mortality for the total number of controls must not exceed 10% at the end of the test;
- the LD_{50} of the toxic standard meets the specified range.

Statistical calculations
Descriptive statistics.

II. RESULTS AND DISCUSSION

A. FINDINGS
The test solution containing a concentration of 100 µg a.s./bee was administered on the ventral part of the thorax of the bees. A summary of the mortality is provided below.

Table B.9.3.1.1.2-5: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in a contact toxicity test

Dose [µg a.s./bee]	Mortality [%]		
	24 h	48 h	72 h
Control (Acetone)	0.00	0.00	0.00
100	0	0	0

B. OBSERVATIONS

No mortality of bees was observed during the 72 hours test period for the test concentration of 100 µg a.s./bee. In addition, no paralysis was observed in the test item group and the control group during the 72 hours test period.

Deviations according to the current guideline OECD 214 (1998):

- Mortality observation was not assessed at 4 hours
- Humidity was lower than the expected range: 34-40% instead of 50-70%
- Test extended to 72h with no rising of mortality of 10 %. Additional assessment in regards to guideline requirement.
- Water control was not setup.

These deviations are not expected to have a negative impact on the validity of the study.

All validity criteria according to OECD 214 were fulfilled, since the average mortality in the control group did not exceed 10% (actual value: 0%) and the 24-hour LD_{50} of the toxic standard meets the standard of less than 1.0 µg a.s./bee based on historical data (actual value: 0.4 µg a.s./bee)..

In an contact toxicity test, glyphosate had no effects on mortality of honey bees at concentrations of up to and including 100 µg a.s./bee.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate was tested in an acute contact toxicity test on honey bees. The LD_{50} (72 h) was >100 µg a.s/bee.

The study is considered valid so LD_{50} >100 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

The test substance was applied on the ventral part of the thorax.
Toxic reference : LD50 = 0.4 µg parathion 25%/bee (no recommended range for parathion in OECD 214), RMS considers that reference toxic performed well.
RMS agrees with the applicant that the deviations mentioned above should not have an impact on the outcome of the study.

This study is valid.

The toxicity of glyphosate was tested in a contact toxicity test on honey bees.
contact toxicity test:
LD50 (72 h) >100 µg a.s./bee

Data point	CA 8.3.1.1.2/007
Report author	[redacted]
Report year	1972
Report title	The acute contact and oral toxicities of CP67573 and MON2139 to worker honey bees
Report No	HU85X094
Document No	-
Guidelines followed in study	Working Document 13 produced by the UK Pesticide Safety Precautions Scheme
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 214 (1998):* *Major:* *- Mortality in the control was >10% at test termination* *Minor:* *- Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group* *- No additional solvent control was tested* *- Duration of starvation was not reported* *- Mortality observation was not assessed at 4 hours.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	No, GLP was not compulsory at the time the study was performed.
Acceptability/Reliability (RMS):	Invalid

* Two test materials were assessed in this study; namely CP67573 and MON2139 (a 36% w/v formulation). MON2139 contains a surfactant that is not present in the representative formulation for the Annex I renewal. This summary therefore only contains information on CP67573 (glyphosate technical).

Executive summary

The contact toxicity of CP67573 (glyphosate technical) to young adult worker bees (*Apis mellifera* L.) was determined in a limit tests performed at a nominal dose of 100 µg CP67573/bee. The test comprised 10 replicate mesh cages, each containing 10 bees. In a parallel test, honey bees were exposed to a reference item in a dose response test using dimethoate at concentrations ranging from 0.048 to 0.117 µg dimethoate/bee. In both tests, the test substance was applied as 1.0 µL drops onto the ventral thorax of CO_2 anaesthetised bees, dissolved in 50% acetone. Control groups consisting of 2 cages of 10 bees were included alongside each of the tests. Assessments of mortality were conducted after 24 and 48 hours. The validity criteria according to OECD guideline 214 were not fulfilled as mortality in the control was > 10% at test termination.

In the 100 µg CP67573/bee treatment group, at 24 and 48 hours, there was 8% and 38% mortality, with corresponding mortality in the control group of 5% and 15% respectively.

This resulted in overall control corrected mortality levels of 3 and 27% achieving a 48 hour LD_{50} of >100 µg CP67573/bee.

The study is considered invalid so endpoints cannot be used for risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item: CP67573 (technical active ingredient)

Description: Not stated

Lot/Batch #: No batch details presented in report

Purity:	Not stated
Density:	Not stated
Vehicle of test material and/or positive control:	Vehicle: 50% acetone Positive control: Dimethoate
Test organisms:	
Species:	Honey bee (*Apis mellifera*)
Age:	Young adult worker bees
Source:	Experienced apiarist in Huntingdonshire, U.K
Diet/Food:	Bees were fed with 20% sucrose
Acclimatisation:	Not reported
Environmental conditions:	
Temperature:	26 – 27°C
Relative humidity:	Not reported
Photoperiod:	Not reported
Experimental dates:	Not reported

B. STUDY DESIGN

Experimental treatments
Honey bees were exposed topically to CP67573 in a limit test conducted at 100 µ test item/bee, in nylon coated 2 mm wire mesh tubes, with 11.5 cm high and 3.5 cm in diameter, closed by corks at both ends. In the contact toxicity test, CP67573 was dissolved in 50% acetone and was applied as 1.0 µL droplets (containing 100 g test item/L) to the ventral thorax of CO_2-anesthetised bees using a micro-applicator. There were 10 cages per test item treatment, with two control cages containing 10 worker bees each. A reference item dose-response test (dimethoate) was conducted in parallel, at five test rates between 0.13 and 0.29 µg test item/bee, with two cages of ten bees per treatment and control group.

Mortality in the test or reference item treatment groups, were corrected for control mortalities using Abbot's correction, to give overall control corrected levels of mortality, on which the endpoint LD_{50} values were based.

Observations
Mortality was recorded 24 and 72 hours after test initiation.

Statistical calculations
Descriptive statistics; LD_{50} for dimethoate were obtained by graphical interpolation on probability/log paper, confidence limits were calculated according to Litchfield & Wilson (1949).

II. RESULTS AND DISCUSSION

A. FINDINGS
A summary of the mortality results is provided below.

Table B.9.3.1.1.2-6: Toxicity of glyphosate to honey bees (*Apis mellifera* L.) in a contact toxicity test

Endpoints (48 h)	CP67573 [µg a.s./bee]
LD_{50} contact	>100

Table B.9.3.1.1.2-7: Contact toxicity of CP67573 to honey bees (*Apis mellifera* L.)

Exposure	Mortality [%]		Corrected mortality [a] [%]
	Control	100 µg a.s./bee	
contact (24 h)	5	8	3
contact (48 h)	15	38	27

B. OBSERVATIONS

In the test with CP67573, the corrected bee mortality did not reach or exceed 50% (max mortality was 27%), resulting in overall control corrected mortality levels of 3 and 27% at 24 and 48 hour respectively, achieving a 48 hour LD_{50} of >100 µg CP67573/bee.

In the reference item test with dimethoate, a 48 hour contact exposure LD_{50} value of 0.16 µg dimethoate/bee (95% C.I. of 0.14 - 0.19 µg dimethoate/bee) was observed.

Deviations according to the current guideline OECD 214:

- Only 2 replicates (10 replicates only for the highest concentration tested) per treatment group
- No additional solvent control was tested
- Duration of starvation was not reported
- Mortality observation was not assessed at 4 hours.

These deviations are not expected to have a negative impact on the validity of the study.

- Mortality in the control was >10% at test termination.

This deviation has a negative impact on the validity of the study.

The validity criteria according to the OECD guideline 214 were not fulfilled as mortality in the control was >10% at test termination.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The toxicity of glyphosate technical (CP67573) was tested in an acute contact toxicity test on honey bees. The LD_{50} (48 h) was >100 µg a.s./bee. The contact LD_{50} for honey bees exposed to MON2139 were determined to be >100 µg a.s./bee.
The study is considered invalid so endpoints cannot be used for risk assessment purposes.

Assessment and conclusion by RMS:

RMS notes that this study was used but not reassessed in the previous RAR 2015 (this study was already used in the 2001 EU evaluation of the substance).
This study was not conducted under GLP (GLP was not compulsory at the time the study was performed).
RMS also notes that solvent control was necessary for contact test and was not conducted. RMS also agrees with the applicant, that control mortality exceeded the validity criteria.
This study is not valid.

Data point:	CA 8.3.1.1.2/008
Report author	[redacted]
Report year	2017
Report title	MON 0139: Acute Oral and Contact Toxicity to the Bumble Bee, *Bombus terrestris* L. under Laboratory Conditions.
Report No	S16-06634
Document No	-
Guidelines followed in study	Based on the proposal for new OECD Guidelines: Bumblebee, acute oral toxicity test (2016) and Bumblebee, acute contact toxicity test (2016)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 246 (2017):* *Minor:* *- analytical verification of dose is missing, however this was not a requirement at the time of study conduct.*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary
Bumblebees (*Bombus terrestris*) were exposed to MON 0139 via contact administration, i.e. cuticular absorption following the application of a droplet to the dorsal body surface of a solution in deionised water. Adult bees were treated with 62.5, 125, 250, 500 and 1000 µg test item/bumble bee. Mortality was recorded 4 and 24 hours after application and thereafter at 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. No mortality was recorded at the end of the test in the 62.5, 250, 500 and 1000 µg test item/bumble bee treatment groups, however, 3.3% mortality (one dead bee) was observed in the 125 µg test item/bumble bee treatment.
The 48 hours contact LD_{50} (Lethal Dose causing 50% mortality) for MON 0139 was determined to be > 1000 µg test item/bumble bee (equivalent to >461 µg a.e./bumble bee). The NOED for mortality after 48 hours was determined to be 1000 µg test item/bumble bee (equivalent to 461 µg a.e./bumble bee). The study was considered valid as there was no mortality in the control group and in the toxic reference group (dimethoate at 13 µg a.s./bumble bee) 100% mortality was observed.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Test item:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (a.e.); 574.4 g/ml
Description:	liquid/slightly yellow
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.2460 g/cm^3

Treatments

Test rates:	Target doses: 62.5, 125, 250, 500 and 1000 µg test item/bumble bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./bumble bee
Control:	Deionised water
Toxic standard:	BAS 152 11 I (dimethoate, analysed 405.2 g a.s./L) 13 µg a.s./bumble bee

Test organisms

Species:	*Bombus terrestris* L. (Hymenoptera: Apidae)
Source:	From healthy colony owned and maintained by Biobest Belgium, Ilse Velden 18, 2260 Westerlo, Belgium.
Food:	50% w/v aqueous sucrose solution

Test design

Test cage description:	Nicot cages
Replication:	30
No. of bees/arena:	1
Duration of test:	48 hours

Environmental conditions

Temperature:	24.8 – 25.3°C
Humidity:	50.9 ± 60.4 %
Photoperiod:	Darkness (except during application and observations)

Experimental dates: 10 April - 13 April 2017

B. STUDY DESIGN

Experimental treatment

Adult worker bumblebees (*Bombus terrestris*) were exposed to MON 0139 via two routes of administration: (1) contact, i.e. cuticular absorption following the application of a droplet to the dorsal body surface of a solution in deionised water. To immobilise the bees during the course of treatment, they were anaesthetised using short bursts of CO_2.

Bumblebees were treated with one 2 µl drop of the test solution, control or toxic standard applied to the dorsal surface of the thorax using a micro applicator. The bumblebees were returned to the test unit, allowed to recover and kept in the CE room with a continuous supply of 50% w/v aqueous sucrose solution.

Assessments

Mortality was recorded 4 and 24 hours after application (after application in the contact toxicity test) and thereafter at 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. In the reference item group, behavioural assessments were not conducted as it was assumed that moribund and affected bumble bees of the reference item group would die by the end of the test.

Statistics

For the statistical evaluation the statistics program ToxRat professional, Version 3.2.1 was used. Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment groups in the contact toxicity test and to determine the NOED based on mortality.

The LD_{50} with 95% confidence limits could not be calculated in the contact toxicity test since the observed mortalities were below 50% in all test item groups.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.3.1.1.2-8: Summary of contact acute toxicity of MON 0139 to the bumblebee

MON 0139	Contact toxicity test	
	[µg test item/bumble bee]	[µg a.e./bumble bee]
LD_{50} (24 h)	>1000	>461
LD_{50} (48 h)	>1000	>461
NOED (48 h)	≥1000	≥461

B. OBSERVATIONS

In the control group treated with deionised water, no mortality occurred during the 48 hours test period. In the test item treatment group, no mortality was recorded at the end of the 48 hours test period in the 62.5, 250, 500 and 1000 µg test item/bumble bee treatment groups. 3.3% mortality was observed in the 125 µg test item/bumble bee treatment groups after 48 hours (corresponding to 1 dead bumble bee). No behavioural abnormalities were recorded during the 48 hours testing period at any target dose.

Deviations according to the current guideline OECD 246 (2017):

- analytical verification of dose is missing, however this was not a requirement at the time of study conduct.

Validity criteria

The study is considered valid since the control and reference item validity criteria were met:
The mean control mortality was ≤10% at the end of the test (actual 0% mortality)
The mean reference item mortality was ≥50% at the end of the test (actual 100% mortality)

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 hours contact LD_{50} for MON 0139 was determined to be >1000 µg test item/bumble bee, equivalent to >461 µg a.e./bumble bee. The NOED for mortality after 48 hours was determined to be ≥1000 µg test item/bumble bee, equivalent to ≥461 µg a.e./bumble bee.
The study is considered valid so LD_{50} >461 µg a.e./bumble bee and NOED ≥461 µg a.e./bumble bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

New study.
Test item: MON 0139 containing glyphosate IPA salt (46.1% acid equivalent (w/w).

RMS notes that analytical verification of dose is missing (this was not a requirement at the time of study conduct). However, this is not part of the validity criteria of the OECD 247 guideline. Moreover, analytical verification is not a requirement for other acute tests on bees (OECD 213, 214). Thus RMS proposed to consider the results as reliable for risk assessment.

This study is valid.
48h Contact – LD50 > 1000 µg MON 0139/bumble bee (equivalent. 461 µg glyphosate acid/bumble bee)

Data point	CA 8.3.1.1.2/009
Report author	[redacted]
Report year	2017
Report title	MON 0139: Acute Contact Toxicity to the Solitary Bee, *Osmia bicornis* under Laboratory Conditions
Report No	S17-00083
Document No	-
Guidelines followed in study	Based on OEPP/EPPO 170 (4) (2010), OECD 214 (1998) and the minutes of the ICPPR Non-Apis bees workshops (2014, 2015, 2016 and 2017)
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*No specific test guideline available.*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
Solitary bees (*Osmia bicornis*) were exposed to MON 0139 by topical application to the thorax following an adapted version of OECD 214. A hand operated micro-applicator was used for contact application of the treatment groups. Adult bees were treated with 62.5, 125, 250, 500 and 1000 µg glyphosate/bee. Three replicate cages each containing 10 bees each were used. Mortality was recorded 4 hours after application and thereafter at 24 hours and 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. Mortality in all glyphosate treated groups was low and did not exceed 6.67% 48 hours after treatment. The 48 hours contact LD_{50} for MON 0139 was determined to be >1000 µg test item/bee (equivalent to >461 µg a.e./bee). The NOED for mortality after 48 hours was determined to be 1000 µg test item/bee (equivalent to 461 µg a.e./bee). The study was considered valid as there was no mortality in the control group and the toxic reference group (dimethoate at 10 µg a.s./bee) 86.7% mortality was observed.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material

Test item:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (a.e.); 574.4 g/ml
Description:	liquid / slightly yellow
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.2460 g/cm^3

Treatments

Test rates:	62.5, 125, 250, 500 and 1000 µg test item/bee, equivalent to 28.8, 57.6, 115, 231 and 461 µg a.e./ bee
Control:	Deionised water
Toxic standard:	BAS 152 11 I (dimethoate, analysed 405.2 g a.s./L) 10 µg a.i./bee
Administration:	Topical application in the torax of 2 µL droplet of the application solution with a hand operated micro-applicator

Test organisms

Species:	*Osmia bicornis* (Linnaeus) (Hymenoptera: Apidae)
Source:	Commercial supplier (WAB-Mauerbienenzucht, Sonnentauweg 47, 78467 D-Konstanz, Germany)
Food:	50% w/v aqueous sucrose solution containing 0.1% anise oil

Test design

Test cage description:	Plastic boxes 13 x 17 cm, height: 6cm
Replication:	3
No. of bees/arena :	10
Duration of test:	48 hours

Environmental conditions

Temperature:	Target: 19.2 – 20.3 °C Exposure: 19.1 – 20.4 °C
Humidity:	Target: 50 – 70% Exposure: 64.4 ± 79.4[1] % [1] Deviations ≥2 hours without impact on the outcome of the study
Photoperiod:	16 hours light : 8 hours dark

Experimental dates: 10 May to 12 May 2017

B. STUDY DESIGN

Experimental treatments

Solitary bees were exposed to MON 0139 by topical application to the thorax. A hand operated micro-applicator was used for application of the treatment groups. The application amount was 2 µL/bee. After anaesthetising the bees by cooling for ~ 1 hour in the refrigerator (~ 10°C) the 2 µL droplet of the application solution was applied individually to the dorsal side of the thorax of each bee. After the application, the bees were returned to the test units, allowed to recover and were fed with a continuous supply of 50% w/v aqueous sucrose solution with anise oil (0.1%). Anise oil was used to attract the bees to the food source (phagostimulant).

Assessments

Mortality was recorded 4 hours after application and thereafter at 24 hours and 48 hours (± 30 min). Behavioural abnormalities such as symptoms of poisoning in comparison to the control were recorded at each observation interval. In the reference item group, behavioural abnormalities assessments were not conducted as it can be assumed that moribund and affected bees of the reference item group died by the end of the test.

Statistics

Multiple Fisher's exact test with Bonferroni-Holm adjustment (one-sided greater, $\alpha = 0.05$) was used to

evaluate whether there are significant differences between the mortality data of the control and the test item treatment group and to determine the NOED based on mortality. The LD_{50} with 95% confidence limits could not be calculated since the observed mortalities were below 50% in all test item groups. Statistical calculations were made by using the statistical program TOXRAT Professional 3.2.1.

II. RESULTS AND DISCUSSION

A. FINDINGS

In the control group treated with deionised water no mortality occurred during the 48-hour test period. After the 24 hour assessment two bees escaped through a hole in the lid of one cage of the control group. As none of the remaining bees showed any effects, and all the remaining bees in the control group survived the impact was deemed minor and the study objective was still achieved.

Table B.9.3.1.1.2-9: Summary of contact acute toxicity of MON 0139 to solitary bee

MON 0139	**Contact toxicity test**	
	[µg test item/bee]	**[µg a.e./bee]**
LD_{50} (24 h)	>1000	>461
LD_{50} (48 h)	>1000	>461
NOED (48 h)	≥1000	≥461

B. OBSERVATIONS

Mortalities of 0.0, 0.0, 3.3, 6.7 and 6.7 % were recorded at the dose levels of 62.5, 125, 250, 500 and 1000 µg product/bee at the end of the 48-hour test period, respectively. No exceptional behavioural abnormalities were recorded throughout the test (one affected bee at the dose level of 62.5 µg test item/bee 48 hours after start of exposure).

Validity criteria

There was no bee mortality in the control group over the 48-hour duration of the test. In the reference item group of the contact toxicity test (deionised water containing dimethoate), 86.7% mortality was observed at the end of the 48 hours test period. Consequently, validity criteria for both control (average mortality ≤ 20%) and reference item mortality (mean mortality ≥ 50%) were met and the test was considered valid.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 48 hours contact LD_{50} for Solitary Bee, *Osmia bicornis* exposed to MON 0139 was determined to be >1000 µg test item/bee, equivalent to >461 µg a.e./bee. The NOED for mortality after 48 hours was determined to be ≥1000 µg test item/bee, equivalent to ≥461 µg a.e./bee.

The study is considered valid so LD_{50} >461 µg a.e./bee and NOED ≥461 µg a.e./bee can be used for risk assessment purposes.

Assessment and conclusion by RMS:

New study.

Test item: MON 0139 containing glyphosate IPA salt (46.1% acid equivalent (w/w).

No specific guideline is available for Osmia. The study was conducted in the laboratory based on OEPP/EPPO 170 (4) (2010), OECD 214 (1998) and the minutes of the ICPPR Non-Apis bees workshops (2014, 2015, 2016 and 2017).

RMS considers this study as valid.
48h Contact – LD50 > 1000 µg MON 0139/solitary bee (equivalent. 461 µg glyphosate acid/ solitary bee)

B.9.3.1.2. Chronic toxicity to bees

Data point	CA 8.3.1.2/001
Report author	[redacted]
Report year	2017
Report title	MON 0139: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory
Report No	118401136
Document No	-
Guidelines followed in study	OECD (2016), Proposal for a New Guideline for the Testing of Chemicals. Honey Bee (*Apis mellifera* L.), Chronic Oral Toxicity Test. 10 Day Feeding Test in the Laboratory, OECD Publishing, Paris, February 2016
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from guideline OECD 245 (2017): none*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

To evaluate the chronic effects of the test item on honey bees, a 10 days chronic oral feeding test in the laboratory (dose response test) was performed. Young honey bees were provided with 5 concentrations (256, 640, 1600, 4000, 10000 mg a.s./kg) of the test item treated sugar solutions ad libitum over a period of 10 days. An untreated control and a reference item (BAS 152 11 I; 400 g/L dimethoate) were included in this study. For the study 3 replicates per treatment were used, each consisting of 10 bees per test cage. The number of dead bees in each test replicate was assessed daily until test end (Day 0 – Day 10). Behavioural abnormalities were assessed daily until test end (Day 1 to Day 10). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded**.** The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day. The quantification of the active ingredient glyphosate of the test item MON 0139 in the feeding solutions was performed using HPLC-method with UV-detection indicating actual doses of 5.6, 10.2, 38.6, 98.0 and 179.9 µg a.s./bee/day (corrected for evaporative losses). Ten days following the start of chronic exposure 3.3 and 6.7% mortality occurred in the 10000 and 640 ppm (179.9 and 10.2 µg a.s./bee/day) treatment groups, respectively. No mortality occurred in the other test item treatments (4000, 1600 and 256 mg a.s./kg feeding solution). There was 6.7% mortality in the control (50% w/v sucrose solution). No behavioural abnormalities occurred following treatment with MON 0139 at any time during the trial.

The chronic oral toxicity of MON 0139 was tested over 10 days.

The LC_{50} value (10 days) was >10000 mg a.s./kg feeding solution.

The LDD_{50} value (10 days) was >179.9 µg a.s./bee/day.

The NOEC and NOEDD values (10 days) were 10000 mg a.s./kg feeding solution and 179.9 µg a.s./bee/day, respectively.

The study is considered valid so LDD_{50} >179.9 µg a.s./bee/day and NOEDD of 179.9 µg a.s./bee/day can be used for the risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test Material:	MON 0139
Lot/Batch #:	GLP-1503-23921-T
Actual content of active ingredients:	Glyphosate: 46.1% (w/w) 574.4 g glyphosate IPA salt/L (analytical), according to certificate of analysis
Description:	Slightly yellow liquid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	February 13, 2018
Density:	1.246 g/cm³ (according to Sponsor); 1.24 g/cm³ (according to MSDS)

Treatments

Test rates:	Concentrations: 256, 640, 1600, 4000, 10000 mg a.s./kg feeding solution Nominal target dose per bee/day: 6.4, 16, 40, 100 and 250 µg a.s./bee/day Actual dose per bee/day: 5.6, 10.2, 38.6, 98.0 and 179.9 µg a.s./bee/day
Control:	50% w/v sucrose solution (500 g sucrose/L deionised water)
Toxic standard:	BAS 152 11 I (nominally 400 g dimethoate/L; analytical 405.2 g/L) 1 ppm dimethoate (1 mg dimethoate/kg feeding solution)
Administration:	The bees in each test unit were fed *ad libitum*, via a single syringe (feeder) attached to each test unit with a 50% (w/v) sucrose solution containing the treatments or control

Test organisms

Species:	*Apis mellifera* (Hymenoptera: Apidae)
Source:	Honey bee colonies, disease-free and queen-right, bred by ibacon.
Food:	50% w/v aqueous sucrose solution. On each day of the test, feeder syringe was replaced with a new syringe containing freshly prepared sucrose solution only (control), or containing the test item or reference item as required.

Test design

Test cage description:	Stainless steel chambers
Replication:	3
No. of bees/arena :	10
Duration of test:	10 days

Environmental conditions

Temperature:	32 - 34°C
Humidity:	59 - 72%
Photoperiod:	Darkness (except during observations)

Experimental dates: 20 June 2017 – 04 September 2017

B. STUDY DESIGN

Experimental treatments

To evaluate the chronic effects of the test item on honey bees, a 10 days chronic oral feeding test in the laboratory (dose response test) was performed. Young honey bees were provided with 5 concentrations of the test item treated sugar solutions ad libitum over a period of 10 days. An untreated control and a reference item (BAS 152 11 I; 400 g/L dimethoate) were included in this study. For the study 3 replicates per treatment were used, each consisting of 10 bees per test cage.

Observations

The number of dead bees in each test replicate was assessed daily until test end (Day 0 – Day 10). Behavioural abnormalities were assessed daily until test end (Day 1 to Day 10). Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded**.** The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day.

Analysis

The quantification of the active ingredient glyphosate of the test item MON 0139 in the feeding solutions was performed using HPLC-method with UV-detection.

Statistics

Levels of bee mortality in the test item groups were compared with mortality levels achieved in the control group. Since mortality in all test item treatment groups was < 50% the LC_{50} / LDD_{50} values could not be calculated and are therefore considered to be greater than the highest tested rate/dose (10000 ppm/179.9 µg a.s./bee/day). The NOEC/NOEDD of the test item was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS

Chemical analysis

The analytical recovery rates of the active ingredient glyphosate in the feeding solutions were as follows:

Table B.9.3.1.2-1: Analytical recovery rates

	Recovery rate [%] [1]	
Concentration [2]	**Day 0** [3]	**Day 9** [4]
10000	96	93
256	92	91

[1] Recovery rate of the a.s. in feeding solution [ppm]
[2] Nominal concentration of the a.s. in the feeding solution [ppm]
[3] Day 0 = freshly prepared feeding solution on day 0
[4] Day 9 = freshly prepared feeding solution on day 9

As the recoveries were within 100% ± 20% nominal concentrations were taken when calculating the dose per bee/day (including correction for evaporative loss).

B. OBSERVATIONS

Effects on honey bees

Over the 10 day chronic exposure period, there was 3.3 and 6.7% mortality in the 10000 and 640 ppm (179.9 and 10.2 µg a.s./bee/day) treatment groups, respectively. No mortality occurred in the other test item treatments (4000, 1600 and 256 mg a.s./kg feeding solution). There was 6.7% mortality in the control (50% w/v sucrose solution). Control mortality was not corrected to the mortality values in the test item treatment. The reference item (dimethoate) at a concentration of 1 ppm (1 mg dimethoate/kg feeding solution) corresponding to 0.015 µg a.s./bee/day caused 100% mortality at day 4.

For each treatment group, based on the actual amount of test solutions consumed (corrected for evaporative losses) within each treatment group, the daily mean doses were 179.9, 98.0, 38.6, 10.2 and 5.6 µg a.s./bee/day after 10 days. The maximum nominal dose levels of the test item (250 µg a.s./bee) could not be achieved, because the bees did not ingest the full targeted volume of treated 50% w/v sucrose solution. Food consumption varies among the treatment group. In the highest dose level (250 µg a.s./bee) the food consumption ranges between 103.7 µg a.s./bee (day 7-8) and 229.0 µg a.s./bee (day 9-10). In the other dose levels the pattern of consumption was more consistent. It is known that there is a high variation of food uptake by the bees within this test. Together with the trophallaxis of the bees the mean values at the end of the test (µg a.s./bee/day) should be seen as the relevant reference point.

No behavioural abnormalities occurred following treatment with MON 0139 at any time during the trial.

Table B.9.3.1.2-4: Summary of chronic oral toxicity of glyphosate to the honeybee

Test Organism		***Apis mellifera* L.**	
Exposure		**Oral 10 days chronic exposure**	
Treatment Group	**Concentration [mg a.s./kg]**	**Dose Level [1] [µg a.s./bee]**	**Mortality at day 10 [2] [% Mean]**
Water control	0.0	0.0	6.7
MON 0139	256	5.6	0.0 (n.s.)
MON 0139	640	10.2	6.7 (n.s.)
MON 0139	1600	38.6	0.0 (n.s.)
MON 0139	4000	98.0	0.0 (n.s.)
MON 0139	10000	179.9	3.3 (n.s.)
Reference Item	1.0	0.015	100.0
Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC**	**NOEDD**
> 10000 mg a.s./kg	> 179.9 µg a.s./bee	10000 mg a.s./kg	179.9 µg a.s./bee

[1] mean dose per bee per day; dose measured based on consumed feeding solution adjusted for evaporation
[2] Mortality at study termination 10 days after start of first feeding
Statistic: Mortality: Fisher`s Exact Test, pairwise comparison, one-sided greater, $\alpha = 0.05$
NOEC/NOEDD: was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$). n.s. = no statistical significant difference compared to the control,

Validity criteria

The study is considered to be valid because it meets the criteria of OECD 245:

- the mean mortality of the control was ≤ 15% (6.7% on day 10)
- the reference item mortality was >50% (actual: 100.0% on day 4)

Ten days following the start of chronic exposure 3.3 and 6.7% mortality occurred in the 10000 and 640 ppm (179.9 and 10.2 µg a.s./bee/day) treatment groups, respectively. No mortality occurred in the other

test item treatments (4000, 1600 and 256 mg a.s./kg feeding solution). There was 6.7% mortality in the control (50% w/v sucrose solution). No behavioural abnormalities occurred following treatment with MON 0139 at any time during the trial. The LC_{50} value (10 days) was > 10000 mg a.s./kg feeding solution. The LDD_{50} value (10 days) was > 179.9 µg a.s./bee/day. The NOEC and NOEDD values (10 days) were 10000 mg a.s./kg feeding solution and 179.9 µg a.s./bee/day, respectively.

III. CONCLUSIONS

Assessment and conclusion by applicant:
This chronic oral toxicity study to honey bees (*Apis mellifera* L.) under laboratory conditions provides relevant and reliable endpoints.

The LC_{50} value (10 days) was > 10000 mg a.s./kg feeding solution. The LDD_{50} value (10 days) was >179.9 µg a.s./bee/day. The NOEC and NOEDD values (10 days) were 10000 mg a.s./kg feeding solution and 179.9 µg a.s./bee/day, respectively.

The study is considered valid so LDD_{50} >179.9 µg a.s./bee/day and NOEDD of 179.9 µg a.s./bee/day can be used for the risk assessment purposes.

Assessment and conclusion by RMS:
New study.
Test item: MON 0139 containing 574 g glyphosate IPA salt /L with 46.1% glyphosate acid (w/w)

This study is valid.
10d – LC50 > 10000 mg glyphosate/kg
10d – NOEC = 10000 mg glyphosate/kg

10d – LDD50 > 179.9 µg glyphosate acid/bee/day
10d – NOEDD = 179.9 µg glyphosate acid/bee/day

B.9.3.1.3. Effects on honeybee development and other honeybee life stages

Data point	CA 8.3.1.3/001
Report author	[redacted]
Report year	2020
Report title	MON 0139 - Repeated exposure of honey bee larvae (*Apis mellifera* L.) under laboratory conditions
Report No	19 48 BLC 0068
Document No	-
Guidelines followed in study	OECD (2016) No. 239 and Adaptations based on SCHMEHL et al. (2016).
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviation from OECD 239 (2016) with adaptation according to SCHMEHL et al., 2016: none*
Previous evaluation	No, not previously submitted
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
The chronic effects of MON 0139 (Glyphosate technical in the form of the IPA salt) on honey bee larvae, was evaluated in a repeat dose laboratory dietary exposure test. Honey bee larvae collected from three different colonies, were exposed to MON 0139 administered at a constant concentration dose in the diet, at five doses of 5.1, 12.8, 31.9, 80 and 200 µg a.s./larva (corresponding to 11.0, 27.5, 68.7, 172 and 429 µg product/larva). An untreated control and a reference item (Dimethoate tech.) were also included in the definitive test. Three replicates per treatment, control or reference item group were prepared, each consisting of 12 larvae, using 48 well plates and polystyrene grafting cells. Cumulative mortality of honey bee larvae treated with the test item was assessed daily from Day 4 to Day 8, with cumulative mortality during the pupal phase assessed on day 15. All mortality was compared to the control. The adult emergence rate was assessed on day 22. Sublethal effects were assessed and recorded daily until test end. The level of glyphosate in the diet was measured using a HPLC-method with UV-detection. In the test item groups, larval mortalities on D8 ranged between 0.0 and 8.3 %. Pupal mortalities on D15, ranged between 11.1 and 23.0 % in the test item treatment groups. Total mortalities on D22 ranged between 19.4 and 36.1%. Mortality in the toxic reference was above 50% across all replicates on D8 (69.4%). No sublethal effects (e.g. remaining food or small body size) were observed at the end of the feeding phase and no other observations occurred in any of the test item treatments on D22.
The ED_{50} (successful adult emergence up to D22) was >200 µg a.s./larva, equivalent to an EC_{50} of >1262 mg a.s./kg diet.
The ED_{20} was determined to be 195.7 µg a.s./larva, which is equivalent to an EC_{20} of 1235 mg a.s./kg diet. Values for ED_{10} and EC_{10} were 75.6 µg a.s./larva and 477 mg a.s./kg diet, respectively.
The respective NOED was 80 µg a.s./larva and the corresponding NOEC was 505 mg a.s./kg diet.
The study is considered valid .

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MON 0139
Lot/Batch #: 11494372
Actual content of active ingredients: MON 0139 is a 62% technical solution comprising Glyphosate at 46.5% (w/w); 580 g/L, according to certificate of analysis

Description:	Yellow liquid
Reanalysis/Expiry date:	29 March 2021
Density:	1.2482 g/mL

2. Treatments

Test rates:	Concentrations: 32, 81, 202, 505 and 1262 mg a.s./kg diet Actual dose per larva: 5.1, 12.8, 31.9, 80 and 200 µg a.s./larva
Control:	untreated diet B/C (aqueous sugar solution + royal jelly)
Toxic standard:	Dimethoate tech. (analysed purity: 98.8% w/w) treated diet B/C at a concentration of 48 mg a.i./kg food
Administration:	Each larva was fed with 20 µL of artificial diet A on day 1, with 20 µL of artificial diet B on day 3 and with 30µL, 40µL and 50 µL of diet C on day 4, 5 and 6 respectively.

3. Test organisms

Species:	*Apis mellifera* Subspecies: Buckfast (Hymenoptera: Apidae)
Source:	Honey bee colonies, disease-free and queen-right, reared by Biochem agrar.
Food:	Artificial diets composed of royal jelly and sugar solution according to the guideline requirements. On each feeding day of the test, freshly prepared diets only were administered to control, or containing the test item or reference item as required.

4. Test design

Test cage description:	Crystal polystyrene grafting cells were placed in 48 well plates
Replication:	3
No. of larvae/replicate :	12
Duration of test:	22 days

5. Environmental conditions

Temperature:	34.0 – 34.8°C
Humidity:	D1-D8: 92 - 100%; D8 - D15: 80-82%; D15 - D22: 60-62%
Photoperiod:	Darkness (except during observations)

6. Experimental dates: 16 September 2019 – 20 November 2019

B. STUDY DESIGN AND METHODS

1. Experimental treatments

To evaluate the chronic effects of the test item MON 0139 on honey bee larvae, a laboratory test (dose response test) after repeated exposure was performed. The test item was administered to the larvae at a constant concentration in the diet according to their growth, within a range of five increasing doses spaced by a factor of ≤3 An untreated control and a reference item (Dimethoate tech.) were included in this study. For the study 3 replicates per treatment, control or reference item were used, each consisting of 12 larvae. All test larvae were collected from three different colonies, each representing a replicate.

2. Observations

Number of dead larvae (an immobile larva or one which did not react to contact stimulus was noted as dead), daily on D4 to D8 (larval mortality); number of dead pupae (larvae that had not transformed into pupae) on D15 (pupal mortality). Recording, e.g. of larger amounts of unconsumed food and/or discolourations and/or abnormal behaviour and/or substantially undersized larvae on D8 in order to

support in the interpretation of mortality data. The test ended on D22 (final assessment) and the bees which emerged successfully were counted.

3. Analytical doses verification

Each final diet was sampled in duplicate for analysis and retained directly after diet preparation on each day of use. The test item stock solutions were sampled in parallel as a back-up in case of issues with the final diet analysis. The determination of the active ingredient was conducted by an in-house developed method using HPLC with MS/MS-detection. The analytical method was validated according to SANCO/3029/99 rev. 4.

4. Statistics

Descriptive statistics were carried out; Step-down Cochran-Armitage Test Procedure (one-sided greater, $\alpha = 0.05$) for determination of NOED/NOEC. $ED/EC_{10/20/50}$ values were determined by Logit analysis using linear maximum likelihood regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The analytical recovery rates of the active ingredient glyphosate in the final diets ranged between 86.8 and 111%. As the measured concentrations always ranged between 80 and 120% of nominal, the ecotoxicological endpoints were evaluated using nominal concentrations. Details are presented below:

Table B.9.3.1.3-1: Analytical recovery rates

Sampling Day	Nominal concentration [µg a.s./L]	Nominal concentration [mg a.s./kg]	Measured concentration [mg a.s./kg]	Recovery rate [% of the nominal]
3	5.1	32.3	34.7	107
4			35.5	110
5			33.4	103
6			36.0	111
3	12.8	80.7	81.4	101
4			75.3	93
5			83.1	103
6			76.6	94.9
3	31.9	202	200	99.3
4			175	86.8
5			200	99.3
6			197	97.7
3	80	505	467	92.6
4			477	94.6
5			462	91.6
6			453	89.7
3	200	1262	1098	87.0
4			1186	94.0
5			1316	104
6			1196	94.8

No test item was detected in the control specimen.

B. OBSERVATIONS

On D8, a larval mortality of 2.8% was observed in the control. Pupal mortality (between D8 and D15) was 19.9% in the control. The control group showed a total mortality of 22.2% on D22 (larval

mortality, pupal mortality, and adults not emerged by D22). In the test item groups, larval mortalities on D8 ranged between 0.0 and 8.3%. Pupal mortalities ranged between 11.1 and 23.0% in the test item treatment groups. Total mortalities on D22 ranged between 19.4 and 36.1%. Mortality in the toxic reference (AR) was above 50% across all replicates on D8, being 69.4%.

No sublethal effects, e.g. remaining food or small body size, were observed at the end of the feeding phase and no other observations occurred in any of the test item treatments on D22.

In the final assessment on D22, an adult emergence rate of 77.8% was determined for the honey bees in the control group. In the test item groups the adult honey bees emerged at rates ranging between 63.9% and 80.6% following an application of 200, 80, 31.9, 12.8 and 5.1 µg a.s./larva.

Table B.9.3.1.3-2: Toxicity of MON 0139 to larvae of *Apis mellifera* L. after repeated exposure

	Control	Test item					Tox. Ref.
Nominal concentrations [mg a.s./kg]	0	32	81	202	505	1262	48
Nominal doses [µg a.s./Larva]	0	5.1	12.8	31.9	80	200	7.6
Larval mortality D3 to D8 abs. [%]	2.8	2.8	0	2.8	8.3	2.8	69.4
Larval mortality D3 to D8 corr. [%]	-	0	0	0	5.7	0	68.6
Pupal mortality D8 to D15 abs. [%]	19.9	11.9	11.1	20.2	20.4	23.0	24.4
Pupal mortality D8 to D15 corr. [%]	-	0	0	0.3	0.5	3.8	5.6
Total mortality D3 to D22 abs. [%]	22.2	19.4	25.0	25.0	33.3	36.1	88.9
Total mortality D3 to D22 corr. [%]	-	0	3.6	3.6	14.3	17.9	85.7
Adult emergence rate [%]	77.8	80.6	75.0	75.0	66.7	63.9*	11.1

Results are averages based on 3 replicates, containing 12 larvae each;
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test and reference item treated groups were corrected by control; negative values were set to "0"; calculations were performed with non-rounded values;
CL: confidence limit; abs.: absolute mortality as counted from the results;
* Statistically significant if compared to the control (Step-down Cochran-Armitage Test Procedure)

Table B.9.3.1.3-3: Endpoints

Endpoints	Nominal doses [µg a.s./Larva]	Endpoints	Nominal concentrations [mg a.s./kg]
ED_{50}[2,3]	>200	EC_{50}[2,3]	>1262
ED_{20}[2] (95% CL)	195.7 (83.9 - 456.7)	EC_{20}[2] (95% CL)	1235 (530 - 2881)
ED_{10}[2] (95% CL)	75.6 (38.8 - 147.3)	EC_{10}[2] (95% CL)	477 (245 - 930)
NOED[1]	80	NOEC[1]	505

[1] Step-down Cochran-Armitage Test Procedure; alpha=0.05; one sided greater
[2] Logit analysis using linear max. likelihood regression
[3] Calculated endpoint was beyond the tested range.

Validity criteria

All the validity criteria according to OECD No. 239 were fulfilled as:

- control mortality was ≤ 15% on D8 (actual value 2.8%)

- cumulative mortality in the reference item treatment group was ≥50% on D8 (actual value 68.6% corrected form control)
- adult emergence in the control was ≥70% on D22 ,

The study is reliable and can be considered as valid.

III. CONCLUSIONS

Assessment and conclusion by applicant:
This repeated exposure larval toxicity study with MON 0139 on honey bees larvae (*Apis mellifera* L.) under laboratory conditions provides relevant and reliable endpoints.

The ED_{50} (successful adult emergence up to D22) was determined to be >200 µg a.s./larva, which is equivalent to an EC_{50} of >1262 mg a.s./kg diet. The ED_{20} was determined to be 195.7 µg a.s./larva, which is equivalent to an EC_{20} of 1235 mg a.s./kg diet. Values for ED_{10} and EC_{10} were 75.6 µg a.s./larva and 477 mg a.s./kg diet, respectively. The respective NOED was 80 µg a.s./larva and the corresponding NOEC was 505 mg a.s./kg diet.

The study is considered valid so NOED of 80 µg a.e./larva can be used for risk assessment purposes.

Assessment and conclusion by RMS:
New study.
Test item: MON 0139 containing 580 g glyphosate IPA salt /L with 46.5% glyphosate acid (w/w)

Dimethoate tech. was used as reference item. This choice was not justified. However no significant effect was observed with the test item during pupal phase (pupal mortality similar to control at all doses). RMS then considers dimethoate appropriate in such case. The reference chemical dimethoate showed that the test system and conditions are responsive.

RMS does not agree to use mean values for the validity criteria. Instead, results of each replicate should be used as the guideline indicates that the criteria has to be met “across all replicate”.
Validity criteria (considering the results for each replicate):
Larval mortality in the control ≤ 15% on D8: max value 8.3% for larvae across all control replicates (between D3 - D8);
Adult emergence rate ≥70%: min value 75% across all control replicates (up to D22);
Larval mortality in the reference item treatment group: min value 58.3 % for larvae across all reference replicates (between D3 and D8)

The validity criteria are met across all replicates. Thus the study is valid.

ED10 = 75.6 µg glyphosate acid/larva
EC10 = 477 mg glyphosate acid/kg diet
NOED = 80 µg glyphosate acid/larva
NOEC = 505 mg glyphosate acid/kg diet.

Considering the 10% effects on emergence at the NOED/NOEC, RMS proposed to base the risk assessment on the EC/ED_{10}.

B.9.3.1.4. Sub-lethal effects

Data point	CA 8.3.1.4/001
Report author	[redacted]
Report year	2012
Report title	Glyphosate: Evaluating potential effects on honeybee brood (*Apis mellifera*) development
Report No	V7YH1001
Document No	-
Guidelines followed in study	Oomen *et al.*, 1992
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline Oomen (1992):* *Minor:* *- Some colonies were slightly smaller in terms of the number of brood frames, but this was not considered to have a significant impact on the study.* *- Feeding period was extended up to 5 days. This extension of the feeding period is not considered to have had an impact on the validity of the study.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

A field study was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) when fed directly to honey bee colonies. The IPA salt was selected as the test substance because it is representative of the active substance in glyphosate formulations and the appropriate for this terrestrial study. Three groups of four colonies were treated with 75, 150 and 301 mg a.e./L of glyphosate in 1 litre of 50% w/v sucrose. One group of four colonies was fed with 1 litre 50% w/v sucrose solution only and one group of four colonies was fed with the toxic reference fenoxycarb dispersed in 1 litre of 50% w/v sucrose. Brood cells were marked in each colony (100 cells containing eggs, 100 cells containing 1-2 day old larvae, and 100 cells containing 3-4 day old larvae) up to 24 hours to dosing using the standard acetate overlay method. On day 7 and just prior to expected emergence, the marked brood cells (eggs, young and old larvae) were assessed for mortality and appearance in each test colony. The content of the dead bee traps attached to the colonies was counted daily during brood assessment period. All colonies were assessed within one week prior to the dosing and within week 1, 2 and 3 after dosing. Samples of each concentration of test item treated sucrose solution were taken on the day of dosing. Four to five day old larvae were sampled 4 and 7 days following start of dosing. Both dosing solution and larval samples were analysed for glyphosate content.

Measured glyphosate (a.e.) concentrations in the dosing solutions were within 11% of the nominal doses. Mean measured glyphosate (a.e.) residues in larvae on 4 days were 13, 37 and 53 mg a.e./kg for the nominal dose levels of 75, 150, and 301 mg a.e./L. Mean measured residues after 7 days were reduced with values of 1.7, 3.2 and 4.1 mg a.e./kg for the nominal dose levels of 75, 150, and 301 mg a.e./L. Glyphosate acid was not detected in the control group.

No biologically significant adult mortality was observed in any treatment group. Over a 16 day observation period after dosing, 2.0 dead pupae/colony were observed in the control and 1.3 – 1.8 dead pupae/colony were observed in the glyphosate treated colonies. Overall survival was 85% for marked eggs, 96% for marked young larvae and 96% for marked old larvae in controls and 82-87% for marked

eggs, 87-94% for marked young larvae and 94-95% for marked old larvae in the glyphosate treated colonies.
The overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg a.e./L (nominal) equivalent to 245 mg a.e./kg nominal when considering the density of the sucrose solution and 266 mg a.e./kg actually measured.

The study is considered valid so NOAEL of 301 mg a.e./L can be used in risk assessment purposes.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	MON 0139
Active substance:	Glyphosate isopropylamine salt
Active substance content:	62.27% Glyphosate isopropylamine salt 46.14% glyphosate acid equivalent/L (measured)
Description:	Clear pale yellow liquid
Lot/Batch #:	GLP-1104-21370-T
Vehicle of test material and positive control:	Vehicle: sucrose solution Positive control: Fenoxycarb (750 mg a.s./L)

Test organism:

Species:	*Apis mellifera* L.
Age:	Not stated
Source:	UK national Bee Unit
Acclimatisation:	not required
Test system:	Twenty standardised field colonies housed in a single chamber wooden Smith hive with British standard frames each and headed by queens of similar age. The honey bee colonies contained 12000 – 22500 adult bees and consisted of 0.5-3 frames of brood, 0.5-2 frames of honey and 0-1 frame of pollen.
Crop cultivated:	Not applicable; the test site with no nearby flowering crops and few flowering weeds, Dunnington, York, U.K.
Replication:	4 colonies/treatment and control

Environmental conditions:

Temperature:	3.4 – 46.3 °C
Relative humidity:	0 – 100%
Average wind speed:	4.0 – 13.1 mph
Precipitation:	0.0 – 9.71 mm
Experimental dates:	21 June – 23 August 2011

B. STUDY DESIGN

Experimental treatments

Test system: Twenty standardised honey bee colonies, each equipped with a dead bee trap fitted to the front were used in this study. All colonies were placed on varroa floors and sticky inserts were placed on the trays to trap any fallen mites. Colonies were located on a test site at Dunnington, York and allowed to fly freely, there were no nearby flowering crops and few flowering weeds (clover). Colonies were placed in groups according to treatment and placed at least 20 m apart from each other.

Experimental design: Up to 24 hours prior to dosing, 100 brood cells containing eggs, 100 cells containing 1-2 day old larvae and 100 cells containing 3-4 day old larvae were selected in each colony and marked using the Oomen *et al.* (1992)[4] acetate overlay sheet method.

Test doses: Dose setting was based on measured residues achieved in a glasshouse residues study after spray application onto *Phacelia* plants at 2.88 kg glyphosate a.e./ha. Considering that bee colonies used in the brood study may be up to 50% bigger than those used in the residue study, an additional calculation for the expected total daily intake of glyphosate residues was undertaken assuming that such colonies would collect 9 g pollen and 1944 mL nectar (see table below). Furthermore the determined residue content based on application of 2.88 kg a.e./ha was adjusted to reflect the lower application rate of 2.16 kg a.e./ha.

Table B.9.3.1.4-1: Exposure assessment of a brood study colony to glyphosate under two scenarios used to establish test doses for use in the brood study

Scenario	Daily intake of glyphosate residues in nectar (1944 g nectar/d) [mg]	Daily intake of glyphosate residues in pollen (9 g pollen/d) [mg]	Total daily intake of glyphosate residues [mg]	Uptake over 3 days [mg]	Adjustment from 2.88 kg a.e./ha to 2.16 kg a.e./ha [mg] [7]
Day 1 maximum mean residues (31.3 µg a.e./g in nectar, 574 µg a.e./g in pollen)	60.8 [1]	5.2 [2]	66.0	198	148.5 [3]
Mean residues over days 1-3 (15.5 µg a.e./g in nectar, 310 µg a.e./g in pollen)	30.3 [4]	2.8 [5]	33.1	99.3	74.5 [6]

[1] Derived from 1.944 kg nectar consumed/day × 31.3 mg/kg = 60.8 mg glyphosate a.e.
[2] Derived from 0.009 kg pollen consumed/day × 574 mg/kg = 5.2 mg glyphosate a.e.
[3] Value of 148.5 mg was rounded to 150 mg to achieve the nominal mid-dose concentration in brood study
[4] Derived from 1.944 kg nectar consumed/day × 15.5 mg/kg = 30.3 mg glyphosate a.e.
[5] Derived from 0.009 kg pollen consumed/day × 310.1 mg/kg = 2.8 mg glyphosate a.e.
[6] Value of 74.5 was rounded to 75 mg to achieve the nominal low-dose concentration in brood study
[7] The determined residue content based on application of 2.88 kg a.e./ha was adjusted to reflect the lower application rate of 2.16 kg a.e./ha.

Test item application: Three groups of colonies (i.e. four colonies per group) were treated with glyphosate isopropylamine salt added to 1 litre of 50% sucrose solution to achieve doses of 75, 150, and 301 mg a.e./L and one group was an untreated control, i.e. fed 1 litre 50% sucrose solution, only. In addition, one group was treated with the toxic reference fenoxycarb, dispersed in 1 L of 50% sucrose (750 mg a.s./L). Doses were administered by removing frames of stores from the colonies and placing a 1 litre glass container containing the treatment solution within the brood chamber.

[4] Oomen, P. A., De Ruijter, A., & Van der Steen J. (1992) Method for honeybee brood feeding tests with insect growth-regulating insecticides. Bulletin OEPP/EPPO Bulletin 22, 613-616.

Observations
The content of dead bee traps was counted daily during the brood assessment period. All colonies were assessed within one week prior to dosing and within weeks 1, 2 and 3 after dosing, including counts of the number of combs of adults, brood, stores and pollen as well as behavioural or physical abnormalities. The uptake of each sucrose solution was checked daily and the container removed when empty or after 5 days. On day 7 the marked brood cells (eggs, young and old larvae) were assessed for mortality and appearance. On day 13 brood cells marked as containing old larvae, on day 15 cells previously containing young larvae and on day 16 cells previously containing eggs, were assessed. Cells were uncapped; the bee removed carefully with forceps and the age of bee was assessed, weighed and observed for deformities. The temperature and humidity were recorded continuously using a data logger; local (within 10 km) weather data was also collected.

Residues analysis
Analysis of glyphosate acid in larvae samples was conducted following extraction with acetonitrile:water (1:4, v/v), clean up by solid phase extraction on C18 and derivatisation as FMOC-glyphosate and a second clean up (solid phase extraction on Oasis HLB, methanolic elution) by HPLC-MS/MS. Analysis of glyphosate acid in treated sugar solution samples was conducted following extraction with acetonitrile:water (1:4, v/v), solid phase extraction on Oasis HLB, methanolic elution and derivatisation as FMOC-glyphosate by HPLC-MS/MS. Limit of quantification (LOQ) and limit of detection (LOD) were 1.0 and 0.3 mg/kg, respectively. Freshly prepared test treated sucrose solution samples were retained for analysis. On day 4 and 7, samples of ten 4-5 day old larvae were collected from each colony for residue analysis.

Data analysis
Brood mortality was analysed using a generalised linear model (Logit distribution) and an ANOVA for pupae weight data to determine NOAEL statistically.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: Residues in samples of sucrose treatment solutions were within 11% of nominal doses. The nominal dose of 75 mg glyphosate a.e./L (corresponding to 61 mg glyphosate a.e./kg) was confirmed to be 65.7 mg glyphosate a.e./kg. The nominal dose of 150 mg glyphosate a.e./L (corresponding to 122 mg glyphosate a.e./kg) was confirmed to be 135 mg glyphosate a.e./kg. The nominal dose of 301 mg glyphosate a.e./L (corresponding to 245 mg glyphosate a.e./kg) was confirmed to be 266 mg glyphosate a.e./kg. (Conversion from nominal dose rate in mg a.e./L to nominal dose rate in mg/kg was based on a density of 50% w/v sucrose solution of 1.23 kg/L.)
Residues in larvae sampled from the hive on day 4 and day 7 ranged from 7.9 to 18.4 and below LOQ to 3 mg glyphosate a.e./kg, respectively on the dose 75 mg a.e./L, from 26.3 to 53.2 and 1.9 to 4.9 mg glyphosate a.e./kg, respectively on the dose 150 mg a.e./L and from 33.1 to 82.1 and 3.2 to 6.3 mg glyphosate a.e./kg, respectively on the dose 301 mg a.e./L, confirming that larvae were exposed to the test item provided in the sugar solution and consumed it.

B. OBSERVATIONS

Consumption of treated sucrose solution: The control colonies consumed between 0.625 and 1.0 L of untreated sucrose. In the glyphosate treated colonies at least 3 of 4 colonies consumed the total volume of treated sucrose.

Bee brood assessments:

Table B.9.3.1.4-5: Survival of marked brood exposed to glyphosate isopropylamine salt

Dose rate [mg/L]	**Control**	**75**	**150**	**301**
Mean dose consumed [mg]		**73 ± 2**	**138 ± 12**	**255 ± 46**
7-d old cells marked as eggs [%]	87.3 ± 1.9	84.8 ± 4.0	87.5 ± 2.7	86.2 ± 3.3
16-d old cells marked as eggs [%]	85.0 ± 2.0	82.3 ± 3.3	86.8 ± 2.7	84.2 ± 3.9
7-d old cells marked as young larvae [%]	96.4 ± 3.0	93.5 ± 1.8	91.5 ± 4.3	95.0 ± 1.8
16-d old cells marked as young larvae [%]	95.9 ± 3.1	93.5 ± 1.8	86.5 ± 4.3	90.0 ± 5.4
7-d old cells marked as old larvae [%]	97.0 ± 0.4	96.8 ± 0.5	96.8 ± 1.7	95.3 ± 2.9
16-d old cells marked as old larvae [%]	95.8 ± 1.3	94.8 ± 1.1	94.3 ± 1.0	95.3 ± 2.9

No significant statistical difference in brood development (eggs, young larvae, old larvae) was observed for all glyphosate treatment groups compared to control ($p<0.05$).

Table B.9.3.1.4-3: Pupae weight at final assessment

Dose rate [mg/L]	**Control**	**75**	**150**	**301**
Mean dose consumed [mg]		**73 ± 2**	**138 ± 12**	**255 ± 46**
Pupae marked as eggs [mg]	127.5 ± 0.7	124.7 ± 0.8	126.7 ± 0.6	135.7 ± 0.6
Pupae marked as young larvae [mg]	128.4 ± 0.6	128.3 ± 1.0	124.4 ± 0.8	125.4 ± 0.6
Pupae marked as old larvae [mg]	128.9 ± 0.4	121.2 ± 0.5	122.6 ± 0.5	125.6 ± 0.4

There were no significant effects of the treatment on the mean weight of the exposed pupae. No biologically significant adult mortality was observed in any treatment group. No adverse effects on colonies were observed in any treatment group apart from an apparent decline in the number of bees and brood in the fenoxycarb treated colonies in the later stages of the study.
In the fenoxycarb toxic reference treated colonies, the overall survival of marked cells was 20% for marked eggs, 0% for marked young larvae and 12% for marked old larvae, meeting the validity criterion for the toxic reference (>40% effect on all stages).

Deviations according to the guideline Oomen (1992):

- Some colonies used in the study were slightly smaller in terms of the number of brood frames, but this was not considered to have a significant impact on the study as all were viable

colonies at the start of the study and a sufficient number of brood cells was available for detailed observations.
- Feeding period was extended up to 5 days (commonly consumed within 24 hours). This extension of the feeding period is not considered to have had an impact on the validity of the study.

III. CONCLUSION

Assessment and conclusion by applicant:
A colony feeding study was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) when fed directly to honey bee colonies. The overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg a.e./L (nominal) equivalent to 245 mg a.e./kg nominal when considering the density of the sucrose solution and 266 mg a.e./kg actually measured.

The study is considered valid so NOAEL of 301 mg a.e./L can be used in risk assessment purposes.

Assessment and conclusion by RMS:
Test item: MON 0139, containing glyphosate as IPA salt
62.27% w/w glyphosate IPA salt corresponding to 46.14% w/w glyphosate acid equivalent

The relevance of the tested concentrations (for the purpose of risk assessment) should be assessed in conjunction with the result of a residue trial (study number V7YH1002) conducted with formulation MON 52276. These results are not presented in this report. Please refer to Vol.3 CP, CP 10.3.1.5/001. Then, some assumptions that are made for the calculations of concentrations might be checked (e.g. how the mean consumption data of 9 g pollen and 1944 mL nectar for the colonies were assessed). Nevertheless this will not affect the relevance of the endpoint itself.

The number of brood frames was below the number stated in study plan (3 frames of brood). However there was no large difference between groups. Therefore, this is considered as a minor deviation.

RMS notes that data loggers attached to the hives (so occasionnally in full sun) recorded extreme temperature values (3.4-46.3°C). However this does not seem to have impacted the colonies (control group performed well). Besides, nearby weather station recorded relevant temperatures.

In the 301 mg glyphosate a.e./L group one colony consumed 0.39 L and the other three each consumed 1.0 L.
In the 150 mg glyphosate a.e. /L group one colony consumed 0.67 L and the other three each consumed 1.0 L.
In the 75 mg glyphosate a.e./L group one colony consumed 0.90 L and the other three each consumed 1.0 L.

Residue analysis are reported below:

Table 6 Residue analysis of sucrose solution samples

Test Group	Nominal dose rate mg/L glyphosate a.e.	Nominal dose rate mg/kg glyphosate a.e.	Measured residue mg/kg glyphosate a.e.	% Nominal
O	0	0	nd	-
A	301	245[1]	266	109
B	150	122[1]	135	111
C	75	61[1]	65.7	108

nd: not detected (LOD = 0.3 mg/kg)
LOQ = 1.0 mg/kg
[1] Conversion from nominal dose rate in mg/L to nominal dose rate mg/kg was based on a density of 50% w/v sucrose solution of 1.23 kg/L.

The toxic reference performed well.

This study is valid.

The overall NOAEL for brood development of honey bees was the highest dose tested:

- 301 mg glyphosate acid equivalent/L (nominal),
- 245 mg glyphosate acid equivalent/kg, nominal (considering density of the syrup);
- 266 mg glyphosate acid equivalent/kg, measured.

B.9.3.2. Effects on non-target arthropods other than bees

In accordance with Commission Regulation (EU) No 283/2013 and 284/2013, non-target arthropods studies were conducted with the current representative formulated product MON-52276. Data are presented in Volume 3 CP under point B.9.5.2.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

Data point	CA 8.4.1/001
Report author	[redacted]
Report year	2009
Report title	MON0139 - Sublethal toxicity to the earthworm *Eisenia fetida*
Report No	09 10 48 056 S
Document No	-
Guidelines followed in study	OECD 222 (2004)
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviation from the guideline OECD 222 (2016): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

The effects of MON0139 (glyphosate isopropylamine salt) on *Eisenia fetida* were tested in a 56 days sublethal laboratory test with regard to the parameters mortality, behavioural and pathological symptoms, body weight change and reproduction in OECD soil containing 10% sphagnum peat. The test was conducted with five nominal test concentrations of 30, 50, 100, 500 and 1000 mg test item/kg dry soil, equivalent to an analysed content of 19.1, 31.9, 63.8, 319.1, and 638.1 mg glyphosate isopropylamine salt/kg dry soil, respectively (*i.e*. 14.2, 23.6, 47.28, 236.4, 472.8 mg glyphosate acid equivalent/kg dry soil, respectively). In addition, a control group was exposed to soil mixed with deionised water only.

After 56 days, the test item caused no mortality at the tested concentrations of 30, 500 and 1000 mg MON0139/kg dry soil. 2.5% mortality was observed at 50 and 100 mg MON0139/kg dry soil. No mortality occurred in the control group. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass when compared to the control group.

All validity criteria according to the OECD guideline 222 were fulfilled. The study is valid so EC_{50} > 473 mg a.e./kg dry soil and NOEC = 473 mg a.e./kg dry soil will be used in the regulatory risk assessment for earthworms exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON0139 (glyphosate isopropylamine salt)
Description:	Pale yellow liquid
Lot/Batch #:	A8B60170S0
Purity:	63.81% w/w glyphosate isopropylamine salt (analysed) 62% w/w glyphosate isopropylamine salt (nominal) 47.28% w/w glyphosate acid equivalent (analysed)
2. Vehicle of test material and/or positive control:	Vehicle: deionised water Positive control: Nutdazim 50 FLOW (carbendazim, SC 500), tested in a separate study

3. Test organism:

Species:	Earthworm (*Eisenia fetida*)
Age:	Adults, approx. 3 months old with clitellum
Weight:	304 – 472 mg
Source:	In-house rearing (originally from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Food:	Air-dried and finely ground horse manure
Acclimation period:	Approx. 24 hours in the artificial substrate

4. Environmental conditions:

Temperature:	18.6 – 21.8 ºC
Photoperiod:	16 h light (600 Lux)/ 8 h dark
Soil pH:	6.1 – 6.2 (test start); 6.0 – 6.1 (test termination)
Soil moisture content:	35.1 – 35.2% (test start); 34.6 – 34.8% (test termination)

B. STUDY DESIGN AND METHODS

1. Experimental treatments: A sublethal test was conducted with five nominal test concentrations of 30, 50, 100, 500 and 1000 mg test item/kg dry soil, equivalent to an analysed content of 19.1, 31.9, 63.8, 319.1, and 638.1 mg glyphosate isopropylamine salt/kg dry soil, respectively. In addition, a control group was exposed to soil mixed with deionised water only. The test concentrations were prepared by dispersing an exactly weighed amount of the test item in deionised water (stock solutions) and thereafter diluted to obtain different test concentrations, which were thoroughly mixed with the artificial soil, achieving desired test concentrations with a final nominal water content of 40 - 60% of WHC. The artificial soil substrate was composed of 10% sphagnum peat, 20% kaolin clay, 69.5% industrial quartz sand and 0.5% calcium carbonate. Four replicate test containers (test item) and 8 replicate test containers (control) with 810 g soil (wet weight) and 5 cm soil depth were prepared for each treatment group. 10 adult earthworms were exposed per replicate for 56 days.

As a toxic reference, earthworms were exposed in a separate study to Nutdazim 50 FLOW (carbendazim, SC 500). The results are in line with the OECD requirements (65% and 92% of reduction in the number of juveniles at concentrations of 5 and 10 mg product/ kg dry soil respectively).

2. Observations: At test initiation, individual fresh weight and behavioural responses of earthworms were recorded. Behavioural and pathological symptoms including feeding activity were observed on a weekly basis. Four weeks after test initiation, number of surviving adult earthworms and fresh weight of surviving adult earthworms per replicate were recorded. At test termination (8 weeks after test initiation), number of surviving juveniles per replicate, were observed.

The behavioural and pathological symptoms, including morphological alterations were observed 4 and 8 weeks after test initiation. Water content and pH measurements were performed at test initiation and at test termination. The temperature was continuously recorded throughout the test.

3. Statistical calculations: Fisher's Exact Binomial Test and Dunnett's t-test were used for mean comparison. For statistical evaluation of the biomass change, mean fresh weight of surviving worms was used.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.1-1: Sublethal effects of MON0139 (glyphosate isopropylamine salt) on earthworm

MON0139 [mg test item/kg soil d.w.]		**Control**	**30**	**50**	**100**	**500**	**1000**
Mortality of adult worms after 4 weeks (%)		0	0	2.5	2.5	0	0
Mean biomass change (%)		+40.7	+46.7	+39.8	+41.8	+37.5	+36.3
Mean number of juveniles after 8 weeks		79.0	78.5	83.8	71.8	80.3	74.3
CV %		18.7	19.1	15.0	34.1	28.7	22.1
Change of reproduction compared to control (%)		-	0.6	-6.0	9.2	-1.6	6.0
EC_{50}	Test item (MON0139)	> 1000 mg/kg dry soil					
	glyphosate isopropylamine salt	> 638.1/kg dry soil					
NOEC	Test item (MON0139)	1000 mg/kg dry soil					
	glyphosate isopropylamine salt	638.1/kg dry soil					

B. OBSERVATIONS

The test item MON0139 caused no mortality at concentrations of 30, 500 and 1000 mg MON0139/kg dry soil. 2.5% mortality was observed at concentrations of 500 and 1000 mg MON0139/kg dry soil. No mortality (0%) occurred in the control group. No effects on behaviour (including feeding activity) of the worms were observed during the test. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) when compared to the control.
The validity criteria according to guideline OECD 222 are fulfilled as each replicate (containing 10 adults) has produced ≥ 30 juveniles by the end of the test in the control and the coefficient of variation of reproduction was ≤ 30% in the control. Also, the adult mortality over the initial 4 weeks of the test was ≤ 10% in the control.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate on mortality and reproduction of earthworms were assessed following application of MON0139 under laboratory conditions.

The EC_{50} of MON0139 for earthworm reproduction was determined to be > 1000 mg test item/kg dry soil, corresponding to > 638.1 mg glyphosate isopropylamine salt/kg dry soil. The overall NOEC was determined to be ≥ 1000 mg/kg dry soil, corresponding to 638.1 mg glyphosate isopropylamine salt/kg dry soil, corresponding to ≥ 473 mg a.e./kg dry soil.

The study is valid so EC_{50} > 473 mg a.e./kg dry soil and NOEC ≥ 473 mg a.e./kg dry soil can be used in risk assessment for earthworms exposed to glyphosate IPA salt.

Assessment and conclusion by RMS:
This study is valid.
Artificial soil containing 10% sphagnum peat
Test item mixed into the soil.
Effects are below 10%. Thus no EC10 could be derived.

NOEC for earthworms = 1000 mg MON 0139 /kg dry soil, equivalent to 473 mg glyphosate acid equivalent/kg dry soil.

Data point:	CA 8.4.1/002
Report author	[redacted]
Report year	2000
Report title	A laboratory investigation of the effects of glyphosate and its breakdown product AMPA on reproduction in the earthworm *Eisenia fetida*
Report No	CEMR-1173
Document No	Not available
Guidelines followed in study	ISO 11268-2 (1998)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 222 (2016):* *Major:* *- Test design for NOEC required at least 5 concentrations (only 2 of each in this study) and 8 replicates for the negative control (only 4 in this study).*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Supportive

Executive Summary

The effects of the isopropylamine (IPA) salt of glyphosate and the metabolite aminomethylphosphonic acid (AMPA) on the earthworm *Eisenia fetida* were tested in a 56-day chronic laboratory test with regard to the parameters mortality, development of body weight and reproduction. The test was conducted with

two test concentrations of glyphosate IPA salt (5.76 and 28.79 mg/kg dry soil (equivalent to 4.27 and 21.31 mg glyphosate acid equivalent/kg dry soil) and two test concentrations of AMPA (5.62 and 28.12 mg/kg dry soil) in artificial soil containing 10% peat. Furthermore, a negative and three concentrations of a positive control (Benlate®) were tested.

Only one adult worm died during the test at the lowest concentration of glyphosate IPA salt (5.76 mg/kg dry soil) tested and thus was not considered to be dose-related. Furthermore, no significant difference in body weight change compared to the untreated controls was noted for adult worms exposed to the glyphosate IPA salt or AMPA at any of the concentrations tested in this study.

No significant differences were observed between the mean juvenile production for the untreated control worms and specimens exposed to glyphosate IPA or AMPA at any concentration tested. Similarly, no significant differences were observed between the numbers of unhatched cocoons present at day 56 in the untreated controls and those in both concentrations of glyphosate IPA salt or AMPA.

RMS consideres this study as supportive only.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item 1:	MON 0139
Description:	Clear liquid
Lot/Batch #:	A9C281
Purity:	62% Isopropylamine (IPA) salt of glyphosate (45.9% glyphosate acid equivalent)
Test item 2:	AMPA (aminomethylphosphonic acid)
Description:	White crystalline powder
Lot/Batch #:	PIT-8912-1385-A
Purity:	99.1%

2. Vehicle of test material and positive control:

Vehicle: deionised water

Positive controls: Benlate® (50% w/w benomyl)

Reference item (in a separate study): 2-chloroacetamide

3. Test organism:

Species:	Earthworm (*Eisenia fetida fetida*)
Age:	Adults, 7-10 months old
Weight:	386 - 477 mg (test initiation)
Source:	In-house culture based on a stock of worms obtained from Blades Biological, UK
Food:	Cattle manure
Acclimation period:	Earthworms were acclimatised to the artificial soil for a period of 29 days at 16 – 22.5°C.

4. Environmental conditions:

Temperature:	18 - 22°C
Photoperiod:	16 h light: 8 h dark
Soil pH:	6.38 – 6.96
Soil temperature:	18.4 – 19.6°C
Soil moisture content:	37.9% (60% of the water holding capacity) (test initiation); 29.6 – 31.1% (test termination)

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The test was conducted with two test concentrations of glyphosate IPA salt (5.76 and 28.79 mg/kg dry soil, equivalent to 4.27 and 21.31 mg glyphosate acid equivalent/kg dry soil) and two test concentrations of AMPA (5.62 and 28.12 mg/kg dry soil). The test item was dissolved in deionised water and the solution was mixed with the water used for adjusting the soil moisture to 60% of the water holding capacity. Afterwards, the solution was mixed into the artificial soil substrate (10% peat; 20% clay, 70% silica sand and calcium carbonate to obtain a pH of 5.5-6.5). 1 g cow manure/100 g dry soil was added as feed. Four replicate test containers with 600 g dry soil were prepared for each treatment group. 10 adult earthworms were exposed for 56 days per replicate. Earthworms were fed with manure on day 1, 14, 21 and 28. Soil moisture was adjusted once a week by adding deionised water. A negative control was treated with deionised water only. As positive control, earthworms were exposed to three concentrations of Benlate® (2.66, 5.93 and 13.28 mg/kg dry soil). Temperature and light intensity were recorded daily during the test period. pH and soil temperature were determined at the beginning and the end of the test in one of the replicate vessels at each concentration. Soil moisture content was determined at day 0, 1, 7, 14, 21, 23, 28, 35, 42 and 56. Furthermore, toxicity of 2-chloroacetamide to *Eisenia fetida* was tested in a separate 14 day reference study.

2. Observations:

Mortality and reproduction: The replicates were examined for live and dead adult worms after 28 days at which time all adult worms were removed and the soil was replaced in the vessels. After a further 28 days, the contents of the beakers were examined for juvenile worms and cocoons.

Mean body weights: All surviving earthworms per replicate were weighed as a group and average individual weights were calculated prior to test initiation and at day 28 after application.

3. Statistical calculations: Mean percent changes in weights of live worms at 28 days and mean juvenile production per surviving adult worm at day 56 were tested for significant ($\alpha = 0.05$) inhibition compared to the controls using the Dunnett's Test (one tailed comparison) in the computer program TOXSTAT Release 3.0. The same test, but with a two-tailed comparison, was employed to test for significant differences between mean numbers of un-hatched cocoons because the test substances may have inhibited cocoon production or/and cocoon viability (cocoons may have been produced but unable to hatch). Each set of data was tested for normality before carrying out the parametric multiple comparison procedure using the Chi-square test and the Shapiro Wilks test, the data were also tested for homogeneity of variance using both the Hartley and the Bartletts tests provided in the program TOXSTAT Release 3.0.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.1-2: Summary of the effects of glyphosate IPA salt, AMPA and the positive control Benlate® on *Eisenia fetida*

Treatment [mg/kg dry soil]		Adult worms		Juvenile production (at day 56)		Mean number of unhatched cocoons per surviving worm
		Percentage mortality of adult worms (at day 28)	Mean percent weight change (at day 28)	Mean number of juveniles per surviving worm	Coefficient of variation	
Control		0	+ 22	31.0	10	0.1
Benlate®	2.66	0	+ 23	26.0 *	15	0.1
	5.93	0	+ 12 *	7.8 *	23	2.2 *
	13.28	0	- 24 *	0.0 *	0	0.7
Glyphosate(1) (as IPA salt)	5.76	2.5	+ 14	26.2	25	0.3(N)
	28.79	0	+ 20	28.5	12	0.3(N)
AMPA	5.62	0	+ 24	26.0	3	0.3(N)
	28.12	0	+ 24	29.4	16	0.4(N)

* = statistically (P = 0.05) different from controls.
(1) = glyphosate was tested as the IPA salt,
N = The numbers of unhatched cocoons present at the end of the test in the glyphosate and AMPA treatments were slightly higher than the controls but statistical analysis proved that this was probably due to random chance alone and was probably not due to the presence of glyphosate or AMPA.

B. OBSERVATIONS

Mortality: Only one adult worm died during the test at the lowest concentration of glyphosate IPA salt (5.76 mg/kg dry soil). This was not considered to be dose-related since no mortalities were observed at higher concentrations.

Mean body weight: No significant difference in body weight change compared to negative control was noted for adult worms at any concentration or test item treatment.

Behaviour: No abnormal behaviour when compared to untreated controls was observed for adult worms at any concentration or test item treatment.

Reproduction: No significant differences were observed between mean juvenile production for untreated control worms and worms exposed to glyphosate IPA salt, at any concentration tested. Similarly, for worms exposed to AMPA no significant difference from the negative control was seen in terms of juvenile production. No significant differences were observed between number of unhatched cocoons present at day 56 in negative control and both concentrations of glyphosate IPA salt. Similarly, for AMPA, no significant difference from the control was observed in terms of numbers of unhatched cocoons.

Positive control: The adult worms exposed to 5.93 and 13.28 mg Benlate®/kg dry soil showed a significantly reduced growth when compared to negative control at day 28. A significant reduction in juvenile production compared to negative control was seen for 2.66, 5.93 and 13.28 mg Benlate®/kg dry soil. At 5.93 mg Benlate®/kg dry soil a significantly increased number of unhatched cocoons was observed when compared to the negative control.

Reference study with 2-chloroacetamide: The 14 day LC_{50} was determined at 39.4 mg/kg dry soil (95% confidence limits; 36.0 - 43.1 mg/kg dry soil).

The resulting endpoint values are given below.

Table B.9.4.1-3: Toxicity of Glyphosate IPA salt and AMPA to *Eisenia fetida*

Endpoints		**Test item [mg/kg dry soil]**
LC_{50}	Glyphosate (as IPA salt)	> 28.79
	AMPA	> 28.12
EC_{50}	Glyphosate (as IPA salt)	> 28.79
	AMPA	> 28.12
NOEC	Glyphosate (as IPA salt)	≥ 28.79 (21.31 mg glyphosate a.e./kg dry soil)
	AMPA	≥ 28.12

The following point deviated from the current OECD guideline:

- Test design for NOEC required at least 5 concentrations (only 2 of each in this study) and 8 replicates for the negative control (only 4 in this study).

According to the study authors, this deviation is not expected to have any impact on the study validity in that case.

The validity criteria according to guideline OECD 222 are fulfilled as each replicate (containing 10 adults) have produced ≥ 30 juveniles by the end of the test in the control and the coefficient of variation of reproduction was ≤ 30% in the control. Also, the adult mortality over the initial 4 weeks of the test was ≤ 10% in the control.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The effects of glyphosate and the metabolite AMPA on mortality and reproduction of *Eisenia fetida* after 56 days of exposure were assessed under laboratory conditions.

Glyphosate, tested as glyphosate IPA salt, and the metabolite aminomethylphosphonic acid (AMPA) had no significant effect on growth or reproduction of *Eisenia fetida* after 56 days of exposure at concentrations up to 28.79 mg glyphosate IPA salt/kg dry soil (21.31 mg glyphosate acid equivalent/kg dry soil) and 28.12 mg AMPA/kg dry soil. Therefore, the NOEC was determined to be ≥ 28.79 mg glyphosate IPA salt/kg dry soil (≥ 21.31 mg glyphosate acid equivalent/kg dry soil) and ≥ 28.12 mg AMPA/kg dry soil.

The study is valid so NOEC ≥ 21.31 mg a.e./kg dry soil can be used in risk assessment for earthworms exposed to glyphosate IPA salt and NOEC ≥ 28.12 mg/kg dry soil can be used in risk assessment for earthworms exposed to AMPA.

Assessment and conclusion by RMS:

The study was conducted according to ISO 11268-2 (1998). The validity criteria of OECD 222 (2016) are fulfilled, however the study design is not in line with the recommendations of this latter guideline. Indeed the test design for NOEC requires at least 5 concentrations (at least), but only 2 were used in this study (for both test items). RMS would consider this low number of test concentrations still adequate for a limit test. However, in such case 8 replicates would be required for both control and treated soil and only 4 are available for each test item and concentration.
Besides 8 replicates for the negative control should have been used but there were only 4 in this study.

For this reason, RMS considers the study as reliable with restrictions and supportive only for the purpose of risk assessment.

Artificial soil (as described in IS0 11268-2) containing 10% Great Mills Irish moss peat
Test items were mixed into the soil.
Glyphosate IPA salt and the metabolite aminomethylphosphonic acid (AMPA) had no significant effect on growth or reproduction of *Eisenia fetida* after 56 days of exposure at concentrations up to 28.79 mg glyphosate IPA salt/kg dry soil (21.31 mg glyphosate acid equivalent/kg dry soil) and 28.12 mg AMPA/kg dry soil.

NOEC = 28.79 mg glyphosate IPA salt/kg dry soil (21.3 1 mg glyphosate acid equivalent/kg dry soil)
NOEC = 28.12 mg AMPA/kg dry soil.
Supportive only.

Data point	CA 8.4.1/003
Report author	[redacted]
Report year	2003
Report title	Laboratory determination of the side-effects of aminomethyl phosphonic acid (AMPA) on the reproductive performance of earthworms (*Eisenia fetida)* using artificial substrate
Report No	01-64-077-ES
Document No	-
Guidelines followed in study	OECD draft document (January 2000): Earthworm Reproduction Test – Proposal for a new guideline
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Deviations from guideline 222 (2016): none*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

The aim of the study was to determine the effects of AMPA (aminomethyl phosphonic acid) on the reproduction of earthworms (*Eisenia fetida)* maintained under laboratory conditions on artificial substrate containing 10% sphagnum peat for 56 days. The test was conducted with eight nominal test concentrations, encompassing 58.6, 87.8, 131.9, 198.1, 297.1, 445.5, 668.5 and 1002.5 mg test item/kg dry soil thoroughly mixed into the soil substrate. The water content was adjusted to about 50% of maximum water holding capacity (WHC). Negative control soil was treated with untreated water only. As a toxic reference, earthworms were exposed to carbendazim at concentrations of 1.0, 2.2 and 5.0 mg/kg dry soil. The test comprised four replicates for each test concentration and toxic reference concentration and eight replicates for the control. The adults were exposed to the test item in the artificial soil substrate for four weeks. Thereafter mortality and mean weight of the survivals were observed. The adults were discarded and after additional four weeks of the test units in the climatic chamber the number

of juveniles were assessed.

No test item related mortality was observed up to 1000 mg AMPA/kg dry soil.

The NOEC based on biomass deviation was determined to be 297.1 mg AMPA/kg dry soil and the NOEC based on reproduction was determined to be 198.1 mg AMPA/kg dry soil. The EC_{50} was 562.7 mg AMPA/kg soil. A NOEC of 131.90 mg test item/kg dry soil was suggested for the parameter biomass and number of juveniles. The study is considered valid so EC_{50} of 562.7 mg/kg dry soil and NOEC of 131.9 mg/kg dry soil will be used for risk assessment of earthworms exposed to AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethyl phosphonic acid)
Description:	White powder
Lot/Batch #:	A0164351
Purity:	99.7% (analysed)

2. Vehicle of test material and positive control: Vehicle: water
Positive control: Carbendazim (99.6%)

3. Test organism:

Species:	Earthworm (*Eisenia fetida*)
Age:	Synchronized adults, > 2 months
Weight:	300 – 600 mg
Source:	In-house rearing (Phytosafe S.A.R.L, 2, rue Marx Dormory, 64000 Pau, France)
Food:	5 g ground cow manure moisten with 6 mL water once per week (days 1, 7, 14, 21 and 28)
Acclimation period:	Not reported

4. Environmental conditions:

Temperature:	19.0 – 21.5 ºC
Photoperiod:	12 h light (416 - 595 Lux)/ 12 h dark
Soil pH:	Control 6.0 (test start), 6.9 (test termination)
	Test item: 5.7 – 6.0 (test start), 6.3 – 6.8 (test termination)
	Reference item: 6.0 (test start), 6.9 – 7.0 (test termination)
Soil moisture content:	Control 43.9% WHC (water holding capacity, at test termination)
	Test item: 44.3 – 46.2% WHC (at test termination)
	Reference item: 44.6 – 45.9 WHC (at test termination)

5. Experimental work dates: November 12th, 2002 to January 08th, 2003

B. STUDY DESIGN AND METHODS

Experimental treatments

A sublethal test was conducted with eight nominal test concentrations and one untreated water control. The test substance was prepared by dispersing 10.0249 g of the test item in 500 mL water. Thereafter eight samples containing 1.46, 2.19, 3.29, 4.94, 7.41, 11.11, 16.67 and 25.0 mL test solution were

thoroughly mixed into the artificial soil, achieving desired test concentrations of 58.6, 87.8, 131.9, 198.1, 297.1, 445.5, 668.5 and 1002.5 mg test item/kg dry soil, with a final nominal water content of 50% of WHC.

Test units contained 500 g of the oven dried weight artificial soil substrate incorporated into 1.5 to 2 L glass containers, composed of 10% sphagnum peat; 20% kaolinite clay and 70% fine sand, each. Four replicate test containers (test item and reference groups) and 8 replicate test containers (control group) were prepared for each treatment group. 10 adult earthworms were exposed per replicate for 56 days.

As a toxic reference, earthworms were exposed to carbendazim at concentrations of 1.0, 2.2 and 5.0 mg test item/kg dry soil, respectively.

Observations
Four weeks after test initiation, percent mortality and mean weight of the surviving adult earthworms were recorded. At test termination (8 weeks after test initiation), the number of surviving juveniles were determined.
Measurements of pH values were performed at test initiation and at test termination. The soil moisture was recorded at test end. Corresponding percent water holding capacity was calculated. The temperature in the climatic chamber was reported without any detailed information on the respective measurements.

Statistical calculations
For statistical evaluation of the biomass deviation and production of juveniles, F-variance analysis was considered ($\alpha = 0.01$). EC_{50} values including 95% confidence intervals were calculated using Excel calculations. EC_{50} calculations were based on untransformed data due to low confidence of log values.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.1-4: Observed effects of AMPA to *Eisenia fetida*

Concentrations [mg test item/kg dry soil]	Observations		
	Mean mortality [%]	Mean biomass deviation [%]	Number of juveniles [Mean ± SD]
Control			
0.0	0.0	- 9.5	120.6 ± 12.4
AMPA			
58.6	0.0	-11.0	114.8 ± 12.1
87.8	0.0	-10.0	112.5 ± 9.8
131.9	0.0	-11.5	110.0 ± 14.8
198.1	0.0	-16.8	109.0 ± 11.2
297.1	0.0	-11.8	93.8 ± 10.2
445.5	0.0	-22.3	66.8 ± 3.4
668.5	2.5	-32.4	41.0 ± 3.2
1002.5	0.0	-34.2	16.3 ± 6.3
Carbendazim			
1.0	0.0	-9.3	56.3 ± 14.9[a]
2.2	2.5	-12.6	9.5 ± 5.8
5.0	2.5	-33.3	0.3 ± 0.5

SD: standard deviation
[a] Percent reduction of the production of juveniles was slightly higher than 50% initially postulated as a maximum.

The EC_{50}, NOEC and LOEC value are given below based on nominal concentrations.

Table B.9.4.1-5: Toxicity to *Eisenia fetida* exposed to AMPA

Parameter	AMPA [mg/kg dry soil]
Biomass deviation	
NOEC	297.1
LOEC	445.5
Reproduction	
EC_{50} (95 % CI)	562.7 (381.2 – 744.1)
NOEC	198.1
LOEC	297.1

CI= confidence interval

B. OBSERVATIONS

There was no mortality in the control and a single mortality in the 668.5 mg test item/kg concentration of the test item treated group and in the 2.0 and the 5.0 mg test item/kg dry soil concentration of the reference item group.
Mean percent of biomass deviation was -9.5% in the control group. In the test item treatment groups, the loss of biomass was similar to the control, ranging from -10.0 to -11.8% in the concentrations between 58.6 and 297.1 mg test item/kg dry soil, with an exception for the 198.1 mg test item/kg dry soil test item with a higher loss in biomass. The loss of biomass was significantly higher for the treatment concentrations of 445.5, 668.5 and 1002.5 mg test item/kg dry soil compared with the control.

Table B.9.4.1-6: Percent biomass deviation after 28 days of exposure of adult earthworms to AMPA

Concentrations [mg test item/kg dry soil]	Replicates 1	2	3	4	5	6	7	8	Biomass deviation [% ± SD]
Control [%]									
0.0	-9.5	-9.1	-6.0	-10.8	-12.8	-12.9	-4.0	-11.0	-9.5 ± 3.1
AMPA[%]									
58.6	-12.5	-9.5	-8.3	-13.8					-11.0 ± 2.6
87.8	-11.1	-9.8	-9.1	-9.9					-10.0 ± 0.8
131.9	-16.5	-11.4	-12.7	-5.5					-11.5 ± 4.6
198.1	-13.9	-16.3	-21.1	-16.0					-16.8 ± 3.0
297.1	-8.1	-14.7	-17.4	-7.1					-11.8 ± 5.0
445.5	-21.7	-24.8	-19.5	-23.2					-22.3 ± 2.3*
668.5	-35.6	-27.9	-29.2	-36.6					-32.4 ± 4.4*
1002.5	-32.2	-34.4	-36.6	-33.4					-34.2 ± 1.9*
Carbendazim[%]									
1.0	-11.2	-10.4	-6.2	-9.3					-9.3 ± 2.2
2.2	-16.1	-9.7	-19.3	-5.3					-12.6 ± 6.3
5.0	-40.5	-35.0	-29.1	-28.6					-33.3 ± 5.6

SD: standard deviation,
*= statistically significant different from the control according to F-variance analysis.

Mean number of juveniles was 120.6 in the control group, the coefficient of variations was 10.3%. The production of juveniles was significantly reduced for treatment concentrations ranging between 297.1

and 1002.5 mg AMPA/ kg dry soil.

Table B.9.4.1-7: Number of juveniles after 56 days of exposure to AMPA

Concentrations [mg test item/kg dry soil]	Replicates 1	2	3	4	5	6	7	8	Number of juveniles [Mean ± SD]	CV in %
Control										
0.0	127	105	125	112	136	134	104	122	120.6 ± 12.4	10.3
AMPA										
58.6	104	122	128	105					114.8 ± 12.1	10.5
87.8	104	121	121	104					112.5 ± 9.8	8.7
131.9	124	106	119	91					110.0 ± 14.8	13.4
198.1	119	94	107	116					109.0 ± 11.2	10.3
297.1	88	109	90	88					93.8 ± 10.2*	10.9
445.5	64	71	64	68					66.8 ± 3.4*	5.1
668.5	45	39	38	42					41.0 ± 3.2*	7.7
1002.5	18	9	24	14					16.3 ± 6.3*	39.0
Carbendazim										
1.0	49	74	62	40					56.3 ± 14.9[a]	26.5
2.2	8	18	7	5					9.5 ± 5.8	61.1
5.0	0	1	0	0					0.3 ± 0.5	200

SD: standard deviation; CV= Coefficient of variation

*= statistically significant different from the control according to F-variance analysis

[a] Percent reduction of the production of juveniles was slightly higher than 50% initially postulated as a maximum.

Validity of the test according to the current OECD guideline:

- Control mortality < 10% (achieved: 0.0%)
- Production of juveniles in the control > 30 per unit (actual values ranging from 104 to 136)
- Coefficient of variation of reproduction in the control ≤ 30% (achieved: 10.3%)

Therefore, all validity criteria according to guideline OECD 222 are fulfilled.

Moisture content was not monitored throughout the test as requested by the test guideline. However, moisture was in an acceptable range at the end of the test and control criteria passed. Therefore, this is only a minor deviation and has not affected the integrity of the study.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The NOEC based on biomass was determined to be 297.1 mg AMPA/kg dry soil and the NOEC based on reproduction was determined to be 198.1 mg AMPA/kg dry soil. The EC_{50} was 562.7 mg AMPA/kg soil.

Statistical re-evaluation was performed by the RMS* (ToXRatPro, Version 2.10) in the RAR 2015. Percent biomass deviation at the end of the exposure period of the adults were re-analysed. Treatments were compared by the t-test procedure after Williams. Significance was $\alpha = 0.05$.

Table B.9.6-9: Biomass change (%) after 28d of exposure of adult earthoworms to AMPA

	AMPA (mg/kg dry soil)								
No.	control	58.6	87.8	131.9	198.1	297.1	445.5	668.5	1002.5
1	90.5	87.5	88.9	83.4	86.1	91.9	78.3	64.4	67.2
2	90.9	90.5	90.2	88.6	83.7	85.3	75.2	72.1	65.6
3	94	91.7	90.9	87.3	78.9	82.6	80.5	70.8	63.4
4	89.2	86.2	90.1	94.5	84	92.9	76.7	63.4	66.6
Replicates	4	4	4	4	4	4	4	4	4
Mean	91.2	89.0	90.0	88.5	83.2*	88.2*	77.7*	67.7*	65.7*
Std.Dev	2.0	2.6	0.8	4.6	3.0	5.0	2.3	4.4	1.7
CV%	2.2	2.9	0.9	5.2	3.7	5.7	2.9	6.5	2.5

*statistically significant different from the control

Table B.9.6-10: Number of earthworm juvenils after 56 days exposure to AMPA

	AMPA (mg/kg dry soil)								
No.	control	58.6	87.8	131.9	198.1	297.1	445.5	668.5	1002.5
1	116	104	104	124	119	88	64	45	18
2	119	122	121	106	94	109	71	39	9
3	135	128	121	119	107	90	64	38	24
4	113	105	104	91	116	88	68	42	14
Replicates	8	4	4	4	4	4	4	4	4
Mean	120.6	114.8	112.5	110.0	109.0*	93.8*	66.8*	41.0*	16.3*
Std.Dev	12.4	12.1	9.8	14.8	11.2	10.2	3.4	3.2	6.3
CV%	10.z3	10.5	8.7	13.4	10.3	10.9	5.1	7.7	39.0

RMS* changed from 8 to 4 replicate values in the control (taking into account a mean of two values) and reported the biomass deviation as a mean percentage.

A NOEC of 131.90 mg test item/kg dry soil was suggested for the parameter biomass and number of juveniles. The study is considered valid so EC_{50} of 562.7 mg/kg dry soil and NOEC of 131.9 mg/kg dry soil, can be used for risk assessment of earthworms exposed to AMPA.

*RMS of the RAR 2015

Assessment and conclusion by RMS:

The study was conducted according to *OECD draft document (January 2000): Earthworm Reproduction Test – Proposal for a new guideline*. The validity criteria of OECD 222 (2016) are fulfilled, and the study design is in line with the recommendations of this current guideline.

Artificial soil containing 10% peat
Test item was mixed into the soil.

Moisture content was measured only at the end of the test (the test guideline requires a measure at the beginning as well). However, as mentioned above by the applicant, moisture was in an acceptable range at the end of the test and control criteria passed. Therefore, this is considered as minor deviation.

Study authors mentioned that the loss of biomass appeared significantly higher for the 198.1 mg/kg treated group but the 297.1 mg/kg treated group did not confirm the phenomenon. In the RAR 2015, a statistic re-evaluation was performed by the former RMS. A NOEC of 131.90 mg/kg was recalculated on the basis of effects on earthworm biomass and a NOEC for earthworm juveniles production for AMPA was recalculated to 131.90 mg/kg.
RMS supports this reassessment.

56-day NOEC of AMPA for mortality, body weight and the reproduction rate of *Eisenia fetida* = 131.90 mg AMPA/kg dry soil

No EC10 was provided. In accordance to Regulation EU No 283/2013, EC10 and EC20 values should have been provided by the applicant (data gap). This should be done during the process of EU review. This is not critical for risk assessment that can be based on NOEC.

Data point	CA 8.4.1/004
Report author	[redacted]
Report year	2002
Report title	AMPA - Earthworm (*Eisenia fetida*), effects on reproduction
Report No	RRR84121
Document No	-
Guidelines followed in study	DIN ISO 11268-2: 1998: Soil quality – effects of pollutants on earthworms – Part 2: Determination of effects on reproduction
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the guideline OECD 222 (2016):* *Major:* *- Coefficient of variation in the reproduction rate for control was 38% instead of <30% required.* *Minor:* *- 3 test item concentrations were tested instead of at least 5* *- 4 replicates for the negative control used instead of 8* *- Food was added just before application instead of 1 day after application*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Supportive

Executive Summary

In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed for 56 days to three test concentrations of AMPA in artificial soil containing 10% sphagnum peat and observed for mortality, growth, and reproduction. A negative control group was maintained concurrently. Four replicate test chambers were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 0.79, 3.94 and 19.7 mg AMPA/kg dry soil. After 28 days, number and weight of

surviving adult worms was determined. After a further 28 days the reproduction rate was determined by counting the numbers of juvenile earthworms and cocoons in each test vessel.
No mortality was observed in any treatment group. The body weight of the earthworms exposed to AMPA were not statistically different when compared to the control up to and including the highest test concentration of 19.7 mg AMPA/kg dry soil. There were no statistically significant effects on reproduction were observed up to and including the highest test concentration of 19.7 mg/kg dry soil. No behavioural abnormalities were observed in any of the treatment groups.
The no-observed-effect-concentration (NOEC) of AMPA for mortality, growth and reproduction of the earthworm *Eisenia fetida* was found to be 19.7 mg test item/kg dry soil, which was the highest concentration tested.
RMS considered this study as supportive only.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (aminomethyl phosphonic acid)
Description:	White powder
Lot/Batch #:	FA005563
Purity:	99%

2. Vehicle of test material and positive control: Vehicle: demineralised water
Positive control: Derosal flüssig (31.5% carbendazim)

3. Test organism:

Species:	Earthworm (*Eisenia fetida*)
Age:	synchronized adults with clitellum, 4 months
Weight:	300 – 600 mg
Source:	Biologische Bundesanstalt (BBA), Braunschweig, Germany
Food:	Dried litter of stinging nettle and porridge oats
Acclimation period:	2 days in artificial soil under test conditions

4. Environmental conditions:

Temperature:	20 ± 2°C
Photoperiod:	16 h light / 8 hours dark (400 - 800 lux)
pH:	5.45 – 5.57 (test start), 6.03 – 6.30 (test termination)
Water content:	46.11-51.53%

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 207, 10% Sphagnum-peat, air dried, finely ground; 20% kaolin clay, 69% industrial quartz sand and 0.43% calcium carbonate). Four replicate test chambers were maintained in each treatment, with 10 worms in each test chamber. Nominal test concentrations of 0.97, 3.94 and 19.7 mg AMPA/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to about 50% of maximum water holding capacity (WHC) using demineralised water. Negative control soil was treated with demineralised water only.

As a toxic reference, earthworms were exposed in a separate study to Derosal flüssig (31.5% carbendazim).

The adult earthworms were exposed to the test item for 4 weeks; the adult worms were counted, removed and weighed per replicate. The remaining soil was returned to the reproductive test for additional 4 weeks. Thereafter, juveniles were counted. Temperature and relative humidity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.

2. Observations: The adult earthworms were exposed to the test item for 4 weeks, after which the artificial soil was emptied onto a tray and the adult worms were counted, removed and weighed per replicate after they were washed under tap water and dried on filter paper. Missing worms and the earthworms, which failed to respond to gentle stimulation, were considered to be dead.

The number of damaged earthworms (e.g. lack of movement, rigidity, etc.) was assessed at day 28 after application.

Individual weight of the earthworms was recorded at day 28 after application.

Reproduction was recorded 8 weeks after the test initiation as mean number of juveniles per test container and replicate.

3. Statistical analysis: As data for body weight changes and the reproduction were normally distributed and homogeneous, the Dunnett's test was used (multiple comparison, two-sided for weight and one sided smaller for reproduction, $\alpha = 0.05$). NOEC and EC-values for reproduction were determined by regression analysis in an appropriate dose-response function.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.1-8: Effects of AMPA on survival, growth and reproduction of *Eisenia fetida*

Test parameter	Control	AMPA [mg test item/kg dry soil]		
		0.79	3.94	19.7
Mortality (day 28) [%]	0	0	0	0
Weight change (day 28) [%][1)]	-	+10.71	+1.79	+7.14
No. of juveniles (day 56)	60 ± 23	64 ± 23	61 ± 5	68 ± 10
CV [%]	38	36	9	14
Reproduction [%] of control (56 days)[1)]	-	+7	+2	+13

[1)] negative values indicate a decrease, positive values an increase when compared to the control

The LC_{50} and NOEC values are given below based on nominal concentrations.

Endpoints	AMPA [mg test item/kg dry soil]	Reference item [mg/kg]
LC_{50} (28 d)	>19.7	>5.04
$NOEC_{mortality}$ (28 d)	19.7	5.04
$EC_{50,\ biomass}$ (28 d)	>19.7	n.d.
$NOEC_{biomass}$ (28 d)	19.7	1.26
$EC_{50,\ repro}$ (56 d)	>19.7	2.9 (2.60 - 3.23)
$NOEC_{repro}$ (56 d)	19.7	1.26

B. OBSERVATIONS

No pathological symptoms or changes in behaviour of the adult earthworms were noted in any of the test item treatments and the control. During test period, body weights of earthworms in treated and control groups slightly increased or remained at starting level. No mortality was observed in any of the

treatment groups and in the control. Different test item concentrations had no effects on the number of offspring. There was no statistically significant difference between the treated groups and the control.

The LC_{50}-value of the reference test item was determined to be 2.9 mg/kg dry substrate.
Each control replicate containing 10 adults produced ≥ 30 juveniles and adult mortality in the control treatments after four weeks did not exceed 10%. The coefficient of variation for reproduction in control groups was higher than 30% at the end of the test. The validity criteria according to guideline OECD 222 are therefore not considered fulfilled.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The no-observed-effect-concentration (NOEC) of AMPA for mortality, growth and reproduction of the earthworm *Eisenia fetida* was found to be 19.7 mg test item/kg dry soil, which was the highest concentration tested.
However, due to the following deviations, the study is considered invalid and not acceptable for risk assessment:

- 3 test item concentrations were tested instead of at least 5
- 4 replicates for the negative control used instead of 8
- Food was added just before application instead of 1 day after application
- Coefficient of variation in the reproduction rate for control was 38% instead of <30% required.

Assessment and conclusion by RMS:
Test item: AMPA
Artificial soil containing 10% peat
Test item was mixed into the soil.

The study was conducted according to DIN ISO 11268-2: 1998.

The validity criteria of OECD 222 (2016) are not fulfilled, the coefficient of variation for reproduction in control groups was slightly higher than 30% at the end of the test (38%).
In RAR 2015, this deviation was reanalysed and addressed: *Shapiro-Wilk´s Test on Normal Distribution (Number of residues = 16; Shapiro-Wilk´s W = 0.965; p(W) = 0.760) showed that treatment data do not significantly deviate from normal distribution. Based on the pre-selected significance level of 0.05, the Levene test indicates variance homogeneity. The validity criteria according to guideline OECD 222 are therefore still considered fulfilled.*

56-day NOEC of AMPA for mortality, growth and reproduction of the earthworm *Eisenia fe*tida = 19.7 mg AMPA/kg dry soil, highest concentration tested.

However the study design is not fully in line with the recommendations of the current guideline. As highlighted by the applicant, the following deviations may lower the reliability of this study:

- 3 test item concentrations were tested instead of at least 5
- 4 replicates for the negative control used instead of 8
- Food was added just before application instead of 1 day after application
- Coefficient of variation in the reproduction rate for control was 38% instead of <30% required.

Indeed the test design for NOEC requires at least 5 concentrations (at least), but only 3 were used in this study. RMS would consider this low number of test concentrations still adequate for a limit test.

However, in such case 8 replicates would be required for both control and treated soil and only 4 are available for each test item and concentration. Besides 8 replicates for the negative control should have been used but there were only 4 in this study.
For this reason, RMS considers the endpoint reliable with restrictions and supportive only for the purpose of risk assessment.

Data point:	CA 8.4.1/005 also referenced under CA 8.4.2.1/005 and CA 8.5/005
Report author	von Mérey, G. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota
Document No	DOI: 10.1002/etc.3438 E-ISSN: 1552-8618
Guidelines followed in study	OECD 222; OECD 226; OECD 232; OECD 216
Deviations from current test guideline	Earthworm cocoons were not counted, in accordance with OECD 222.
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability (RMS):	Yes/Reliable This publication actually corresponds to the regulatory studies summarized and already assessed by RMS CA 8.4.1/001 [redacted] 2009 Glyphosate IPA salt Eisenia andrei CA 8.4.1/003 [redacted] 2003 AMPA Eisenia fetida CA 8.4.1/001 [redacted] 2010 Glyphosate IPA salt Folsomia candida CA 8.4.1/002 [redacted] 2009 Glyphosate IPA salt Hypoaspis aculeifer CA 8.4.2.1/003 [redacted] 2010 AMPA Folsomia candida CA 8.4.2.1/004 [redacted] 2010 AMPA Hypoaspis aculeifer CA 8.5/001 [redacted] 2014 Glyphosate Nitrogen transformation CA 8.5/004 [redacted] 2010 AMPA Nitrogen transformation Therefore, this publication was not assessed by RMS.

Assessment and conclusion

<u>Assessment and conclusion by applicant</u>:

The aim of the paper was to evaluate potential effects of Glyphosate, Glyphosate salt and AMPA on earthworm, soil mites, springtails and soil micro-organisms.

The studies have been conducted according to recognised guidelines and validity criteria were presented. Test substance information, test organism origin, study designs and toxicity effects were adequately described. The study is considered reliable.

Assessment and conclusion by RMS:

This publication actually corresponds to the regulatory studies summarized and already assessed by RMS
CA 8.4.1/001 [redacted] 2009 Glyphosate IPA salt Eisenia andrei
CA 8.4.1/003 [redacted] 2003 AMPA Eisenia fetida
CA 8.4.1/001 [redacted] 2010 Glyphosate IPA salt Folsomia candida
CA 8.4.1/002 [redacted] 2009 Glyphosate IPA salt Hypoaspis aculeifer
CA 8.4.2.1/003 [redacted] 2010 AMPA Folsomia candida
CA 8.4.2.1/004 [redacted] 2010 AMPA Hypoaspis aculeifer
CA 8.5/001 [redacted] 2014 Glyphosate Nitrogen transformation
CA 8.5/004 [redacted] 2010 AMPA Nitrogen transformation

Therefore, this publication was not assessed by RMS.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

B.9.4.2.1. Species level testing

Data point	CA 8.4.2.1/001
Report author	[redacted]
Report year	2010
Report title	MON0139 – Effects on the reproduction of the collembolans *Folsomia candida*
Report No	09 10 48 057 S
Document No	-
Guidelines followed in study	ISO 11267 (1999)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 232 (2016):* *Minor:* *- 5 replicates were used for the test item treatment groups and the control, instead of 4 in the test item group and 8 in the control* *- 10% sphagnum peat was used instead of 5%* *- 30 g wet weight per test vessel was used instead of 30 g dry weight.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

In a laboratory study the toxicity and reproductive inhibition of Glyphosate isopropylamine salt to *Folsomia candida* was tested. Juvenile springtails, approximately 10 - 12 days old, were exposed to 32, 50, 100, 500 and 1000 µL glyphosate isopropylamine salt/kg dry soil (equivalent to 19, 29, 59, 294 and 587 mg glyphosate acid equivalent/kg dry soil) and to a control with deionised water. A toxic reference (Betosip) was tested in a separate study.
50 springtails (10/ test vessel) per test concentration and control were put in a glass container on artificial soil with incorporated test item and adults and juveniles counted after 28 days.

This study is considered valid. The NOEC is 1000 µL MON 0139/kg soil d.w. (587 mg glyphosate a.s./kg soil d.w.) and EC50 > 1000 µL MON 0139/kg soil d.w. (587 mg glyphosate a.s./kg soil d.w.), highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON0139 Glyphosate isopropylamine salt
Description:	Pale yellow liquid
Lot/Batch #:	A8B60170S0
Purity:	Nominal: 62% w/w glyphosate isopropylamine salt (corresponding to 45.9% w/w glyphosate acid equivalent) Analysed: 63.81 ± 0.29% w/w glyphosate isopropylamine salt (corresponding to 47.28 ± 0.21% w/w glyphosate acid equivalent)
2. Vehicle of test material and positive control:	Vehicle: deionised water Positive control: Betosip (Phenmedipham EC 157 g/L)

3. Test organisms:

Species:	*Folsomia candida* (Willem)
Age:	Juvenile springtails (10 – 12 d old)
Source:	In-house culture originally obtained from Biologische Bundesanstalt (BBA), Berlin, Germany
Diet/Food:	Approximately 2 mg granulated dry yeast at test start and after 14 days

4. Environmental conditions:

Temperature:	20.4 – 21.1 °C
Composition of artificial soil	10% sphagnum peat 20% kaolin clay 0.5% calcium carbonate 69.5% quartz sand Deionised water
Soil water content:	Test start: 34.9 – 35.2% (54.4 – 54.9% of WHC) Test end: 34.5 – 34.7% (53.8 – 54.1% of WHC)
pH:	Test start: 6.01 – 6.08 Test end: 5.79 – 5.91
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	580 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: MON0139 was evaluated for mortality and reproductive reduction in a test with *Folsomia candida* at five application rates of 32, 50, 100, 500 and 1000 µL MON0139/kg dry soil (19, 29, 59, 294 and 587 mg glyphosate acid equivalent/kg dry soil). In addition, a blank control with deionised water and a toxic reference (Betosip) were conducted. Each test item concentration and the control were tested with 50 springtails (10/ test vessel). For each test item concentration and for the

control group 2 test vessels without springtails were provided for measurement purposes. The springtails were put in a glass container (~ 150 mL) containing 30 g (wet weight) artificial soil with the requested test item concentrations and covered with a glass lid for 28 days. Four weeks after introducing the test organisms the parental and juvenile collembolans were counted.

2. Observations: Water content and pH were determined at test start and end. Adults and juvenile springtails were counted at test end.

3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality. Welch-t-test ($p \leq 0.05$) for significance of reproductive reduction. Statistical program: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.2.1-1: Mortality and reproductive reduction of *Folsomia candida* after application of MON0139 in a 28 days laboratory study

Test rate [µL MON0139/kg dry soil]	Test concentration [mg glyphosate a.e./kg dry soil]	Mortality of parental collembolans after 4 weeks [%]	Corrected mortality [1] [%]	Mean number of juveniles after 4 weeks [%]	SD	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	Control	4	-	397.2	56.3	-	14.2
32	19	6	2	355.6	51.0	10	14.3
50	29	6	2	384.6	147.8	3	38.4
100	59	2	-2	344.4	37.4	13	10.8
500	294	0	-4	446.4	89.3	-12	20.0
1000	587	8	4	358.8	43.4	10	12.1

[1] calculated with Abbott 1925

Reference test:
After treatment with the reference item Betosip (Phenmedipham EC 157 g/L) at concentrations of 50, 100, 200 and 400 mg test item/ kg dry soil an EC_{50} of 181.0 mg Betosip/kg dry soil was determined.

B. OBSERVATIONS

No statistically significant effects on parental mortality (Fishers's Exact Binomial Test, $p > 0.05$) or the number of offspring (Welch-t-test, $p > 0.05$) compared to the control was found.

The LC_{50} and EC_{50} values as well as the NOEC are given below based on nominal concentrations.

Endpoints	MON0139 [µL test item/kg dry soil]	Glyphosate acid equivalent [mg a.e./kg dry soil]
NOEC (mortality)	1000	587
NOEC (reproduction)	1000	587
EC_{50} (28 d)	> 1000	> 587

Reference test:
The EC_{50} reproduction with the reference item Betosip (Phenmedipham EC 157 g/L) demonstrated the sensitivity of the test system.

All validity criteria according to OECD 232 were fulfilled, since the mean adult mortality did not exceed 20%, the mean number of juveniles per vessel was ≥ 100 and the coefficient of variation of juveniles was less than 30%.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of glyphosate on mortality and reproduction of *Folsomia candida* were assessed following application of MON0139 under laboratory conditions.

The 28-day EC_{50} was > 1000 µL MON0139/kg dry soil (>587 mg glyphosate acid equivalent/kg dry soil). The NOEC was ≥ 1000 µL MON0139/kg dry soil (≥ 587 mg glyphosate acid equivalent/kg dry soil), the highest tested concentrations, since MON0139 had no negative effect on the test organisms. There were some deviations to the current guideline. However, these deviations did not affect the scientific validity of the study.

The study is considered valid and NOEC ≥ 587 mg a.e./kg dry soil can be used in risk assessment for *Folsomia* exposed to glyphosate IPA salt.

Assessment and conclusion by RMS:

Test item: MON 0139 with nominal content of 62% w/w glyphosate isopropylamine salt (corresponding to 45.9% w/w glyphosate acid equivalent)
Test item was mixed into the soil.

The study was conducted according to ISO 11267 (1999). The validity criteria of OECD 232 (2016) are fulfilled, however the study design is not fully in line with the recommendations of this current guideline.
Deviations from guideline OECD 232 (2016):
- 5 replicates were used for the test item treatment groups and the control, instead of 4 in the test item group and 8 in the control.
RMS considers that 5 replicates in treated groups actually represents an improvement of the study design. Eight control replicates are usually required according to the guideline OECD 232, however, as no apparent effect was observed among treated groups and considering the rather low SD value (on number of juveniles) obtained from control group, RMS considers this deviation acceptable.
- 10% sphagnum peat was used instead of 5%
The impact of such deviation is not clear. As the log Pow of the test item is low, RMS considers that the impact should then be limited. RMS considers the deviation minor.
- 30 g wet weight per test vessel was used instead of 30 g dry weight.
RMS considers this is minor deviation.

RMS notes that a detailed description of the extraction efficiency is lacking.
The number of parental and juvenile collembolans (floating on the surface) was determined by counting adults and juveniles by means of a digital image processing system (LemnaTec Scanalyzer). This is an automated counting technique based on a video camera connected with a frame grabber. By scanning the surface of the container with the digital image processing system, the number of juvenile and adult individuals was quantified using the image processing software from LemnaTec. The study author states that the requirement of the former ISO guideline concerning the precision of the counting method (average error <10 %) was fulfilled, and that the determined overall error of counting amounted to 3.2 %.
OECD 232 requires that the validity includes extraction efficiency of juveniles greater than 95%, e.g. by adding a known number to soil. However no further information could be retrieved in this study report.

Reference test:
Reference item Betosip (Phenmedipham EC 157 g/L) was used to demonstrate the sensitivity of the test system. No specific range of acceptability is given in OECD 232 for this toxic reference, but the EC50 value fulfils the recommendation of ISO 11267 (1999).

This study is considered valid.

NOEC = 1000 µL MON 0139/kg soil d.w. (587 mg glyphosate a.s./kg soil d.w.).
EC50 > 1000 µL MON 0139/kg soil d.w. (587 mg glyphosate a.s./kg soil d.w.), highest concentration tested.

No EC10 or EC20 have been proposed. However this is acceptable in view of the results.

Data point:	CA 8.4.2.1/002
Report author	[redacted]
Report year	2009
Report title	MON0139 – Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Report No	09 10 48 058 S
Document No	-
Guidelines followed in study	OECD 226 (2008)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 226 (2016):* *Minor:* *- Four concentrations of the test item were tested instead of at least five for a NOEC test design.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Valid

Executive Summary
In the laboratory study the toxicity and reproductive inhibition of MON0139 to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 50, 100, 500 and 1000 mg MON0139/kg dry soil (equivalent to 23.64, 47.28, 236.40 and 472.80 mg glyphosate acid equivalent/kg dry soil) and to a control with deionised water. A toxic reference (Perfekthion) was tested in a separate study.
40 mites (10/test vessel) per test concentration and 80 mites per control (10/test vessel) were put in a glass bottle on artificial soil with incorporated test item and adults and juveniles counted after 14 days.
The test item MON0139 caused no statistically significant mortality of adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed.
This study is considered valid. The 4d NOEC is 1000 mg MON 0139/kg soil d.w. (472.80 mg glyphosate a.s./kg soil d.w.) and EC50 > 1000 mg MON 0139/kg soil d.w. (472.80 mg glyphosate a.s./kg soil d.w.), highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	MON0139 (glyphosate isopropylamine salt)
Description:	Pale yellow liquid
Lot/Batch #:	A8B60170S0
Purity:	Nominal: 62% w/w glyphosate isopropylamine salt (corresponding to 45.9% w/w glyphosate acid equivalent) Analysed: 63.81 ± 0.29% w/w glyphosate isopropylamine salt (corresponding to 47.28 ± 0.21% w/w glyphosate acid equivalent)

2. Vehicle of test material and positive control:

Vehicle: deionised water

Positive control: Perfekthion (Dimethoate, EC 400, 422.4 g/L analysed)

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites
Source:	In-house culture originally obtained from Katz Biotech AG, 15837 Baruth, Germany
Diet/Food:	*Tyrophagus putrescentiae* (Schrank) were fed every 2 days, before and during the test

4. Environmental conditions:

Temperature:	19.7 – 21.9 °C
Composition of artificial soil	5% sphagnum peat 20% kaolin clay 0.3% calcium carbonate 74.7% quartz sand Deionised water
Soil water content:	Test start: 18.79 – 20.21% (47.52 – 51.11 % of WHC) Test end: 18.65 – 20.11% (47.17 – 50.87% of WHC)
pH	Test start: 5.9 – 6.2 Test end: 5.3 – 5.4
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	588 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: MON0139 was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at four application rates of 50, 100, 500 and 1000 mg MON0139/kg dry soil (equivalent to 23.64, 47.28, 236.40 and 472.80 mg glyphosate acid equivalent/kg dry soil). In addition, a control with deionised water and a toxic reference (Perfekthion, 422.4 g/L dimethoate) were tested.

Each test item concentration was tested with 40 mites (10/test vessel), while the control group consisted of 80 mites (10/test vessel). For each test item concentration and for the control group 2 test vessels without mites were provided for measurement purposes.

The mites were put in glass bottles with screw tops of 100 mL containing 20 g (dry weight) artificial soil with the requested test item concentrations and closed. Test vessels were opened every two days for food supply and aeration. Two weeks after introducing the test organisms the parental and juvenile mites were counted.

2. Observations: Water content and pH were determined at test start and end. Temperature was recorded continuously. Adult and juvenile mites were counted at test end.

3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality. Dunnett-t-test ($p \leq 0.05$) for significance of reproductive reduction. Statistical program: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.2.1-2: Mortality and reproductive reduction of *Hypoaspis aculeifer* after application of MON0139 in a 14 day laboratory study

Test rate [mg MON0139/ kg dry soil]	Test rate [mg a.e./ kg dry soil]	Mortality of adults after 14days [%]	Corrected mortality [1)] [%]	Mean number of juveniles after 14 days [%]	SD	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	Control	8.8	-	190.5	16.9	-	8.9
50	23.64	10	1.4	176.8	21.7	7.2	12.3
100	47.28	12.5	4.1	173.5	21.5	8.9	12.4
500	236.40	10.0	1.4	182.3	21.4	4.3	11.7
1000	472.80	7.5	-1.4	207.8	13.1	-9.1	6.3

[1)] calculated with Abbott 1925

Reference test:
After treatment with the reference item Perfekthion (Dimethoate, EC 400, 422.4 g/L analysed) at concentrations of 4.1, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil an EC_{50} (reproduction) of 4.9 mg test item/kg dry soil was concluded.

B. OBSERVATIONS

The test item MON0139 caused no statistically significant mortality (Fishers's Exact Binomial Test, $p > 0.05$) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed (Dunnett-t-test, $p > 0.05$).

The EC_{50} value and the NOEC are given below.

Endpoints	MON0139 [mg/kg dry soil]	Glyphosate acid equivalent [mg/kg dry soil]
NOEC	1000	472.80
EC_{50} (14 d)	> 1000	>472.80

Reference test:
The EC_{50} (reproduction) with the reference item Dimethoate EC 400 was in line with the range defined in the guideline to demonstrate the sensitivity of the test system.

All validity criteria according to OECD 226 were fulfilled, as adult mortality in the control treatments did not exceed 20%, the mean number of juveniles per replicates was > 50 at test end and the coefficient of variation of the number of juveniles per replicate was not higher than 30% at test end.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of MON0139 on mortality and reproduction of *Hypoaspis aculeifer* were assessed for 14 days under laboratory conditions.

The 14-day EC_{50} was >1000 mg MON0139/kg dry soil (473 mg glyphosate acid equivalent/kg dry soil). The NOEC was ≥ 1000 mg test item/kg dry soil (≥ 473 mg glyphosate acid equivalent/kg dry soil), the highest tested concentration, since MON0139 had no negative effect on the test organisms.

The study is considered valid so EC_{50} > 473 mg a.e./kg dry soil and NOEC ≥ 473 mg a.e./kg dry soil can be used in risk assessment for *Hypoaspis* exposed to glyphosate IPA salt.

Assessment and conclusion by RMS:

Test item: MON 0139 with nominal content of 62% w/w glyphosate isopropylamine salt (corresponding to 45.9% w/w glyphosate acid equivalent)
Test item was mixed into the soil.

The study was conducted according to OECD 226 (2008). The validity criteria of OECD 226 (2016) are fulfilled, however the study design is not fully in line with the recommendations of this current guideline.
Deviations from guideline OECD 226 (2016):

- Four concentrations of the test item were tested instead of at least five for a NOEC test design. RMS considers this deviation is minor.

About extraction efficiency :
The study author states that the extraction efficiency of the extractor was determined to be 95% in a separate extraction run using vessels containing a known number of juveniles and adult mites kept in untreated test substrate (current guideline OECD 226 requires >90%). However, a detailed description of the extraction efficiency is lacking.

Reference test:
The toxic reference performed well.

This study is considered valid.

14d NOEC = 1000 mg MON 0139/kg soil d.w. (472.80 mg glyphosate a.s./kg soil d.w.).
EC50 > 1000 mg MON 0139/kg soil d.w. (472.80 mg glyphosate a.s./kg soil d.w.), highest concentration tested.

No EC10 or EC20 values have been proposed. However this is not considered necessary in view of the results.

Data point	CA 8.4.2.1/003
Report author	[redacted]
Report year	2010
Report title	AMPA – Effects on the Reproduction of the collembolans *Folsomia candida*
Report No	10 10 48 054 S
Document No	Not available
Guidelines followed in study	OECD 232 (2009) ISO 11267 (1999)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 232 (2016)*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	valid

Executive Summary
In the laboratory study the toxicity and reproductive inhibition of AMPA to *Folsomia candida* was tested. Juvenile springtails, 9-12 days old, were exposed to 30, 54, 97.2, 175 and 315 mg test item/kg dry soil and to a control with deionised water. A toxic reference (100% boric acid) was tested in a separate study. 40 springtails (10/ test vessel) per test concentration and 80 springtails per control (10/ test vessel) were put in a glass container on artificial soil with incorporated test item and adults and juveniles counted after 28 days. No statistically significant effects on parental mortality and number of offspring were observed.
This study is considered valid. The 28d NOEC is 315 mg AMPA/kg dry soil and EC50 > 315 mg AMPA/ kg dry soil, highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solid
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7%

2. Vehicle of test material and positive control: Vehicle: deionised water
Positive control: Reference item: Boric acid (100%)

3. Test organisms:

Species:	*Folsomia candida* (Willem)
Age:	Juvenile springtails (9 – 12 d old)
Source:	In-house culture originally obtained from Biologische Bundesanstalt (BBA), Berlin, Germany
Diet/Food:	Approximately 2 mg granulated dry yeast at test start and after 14 days

4. Environmental conditions:

Temperature:	20.4 – 22.0 °C
Composition of artificial soil	5% sphagnum peat 20% kaolin clay 0.3% calcium carbonate 74.7% quartz sand Deionised water
Soil water content:	Test start: 24.9 – 25.1% (57.8 – 58.2% of WHC) Test end: 24.3 – 25.0% (56.4 – 58.0% of WHC)
Soil pH:	Test start: 5.78 – 5.98 (test start) Test end: 5.60 – 5.78
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	750 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: AMPA at five concentrations, 30, 54, 97.2, 175 and 315 mg test item/kg dry soil, was evaluated for mortality and reproductive reduction in a test with *Folsomia candida*. In addition, a control with deionised water and a toxic reference (100% boric acid) were conducted. Each test item concentration was tested with 40 springtails (10/ test vessel), while the control group consisted of 8 replicates. For each test item concentration and for the control group 2 test vessels without springtails were provided for measurement purposes. The springtails were held in a glass container (~ 150 mL), containing 30 g (wet weight) artificial soil including the requested test item concentrations and covered with a glass lid for 28 days. Four weeks after introducing the test organisms the parental and juvenile collembolans were counted.

2. Observations: Water content and pH values were determined at test start and end. Adults and juvenile springtails were counted at test end as well as physiological or pathological symptoms.

3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality Dunnett-t-test ($p \leq 0.05$) for significance of reproductive reduction Statistical program: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.2.1-3: Mortality and reproductive reduction of *Folsomia candida* after application of AMPA in a 28-day laboratory study

AMPA [mg test item/kg dry soil]	Mortality of parental collembolans after 4 weeks [%]	Corrected mortality [1)] [%]	Mean number of juveniles after 4 weeks [%]	SD	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	6.3	-	931	140	-	15.1
30	5.0	-1	925	107	1	11.6
54	7.5	1	934	49	0	5.2
97.2	2.5	-4	946	112	-2	11.8
175	7.5	1	973	195	-4	20.1
315	2.5	-4	939	201	-1	21.3

[1)] calculated with Abbott 1925

Reference test:
After treatment with the reference item boric acid at concentrations of 44, 67, 97.2, 150 and 225 mg test item/ kg dry soil an EC_{50} of 108.6 mg test item/ kg dry soil.

B. OBSERVATIONS

No statistically significant effects on parental mortality (Fishers's Exact Binomial Test, $p > 0.05$) or the number of offspring (Dunnett-t-test, $p > 0.05$) compared to the control was found.

The LC_{50} and EC_{50} values as well as the NOEC are given below based on nominal concentrations.

Endpoints	AMPA [mg/kg dry soil]
NOEC (mortality)	315
NOEC (reproduction)	315
LC_{50} (28 d)	> 315
EC_{50} (28 d)	> 315

Reference test:
The EC_{50} reproduction with the reference item boric acid was in line the expected result defined in the guideline to demonstrate the sensitivity of the test system (about 100 mg test item/kg dry soil).

All validity criteria according to OECD 232 were fulfilled, since the mean adult mortality did not exceed 20%, the mean number of juveniles per vessel was ≥ 100 and the coefficient of variation of juveniles was less than 30%.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of AMPA on mortality and reproduction of *Folsomia candida* were assessed for 28 days under laboratory conditions.

The 28-day LC_{50} and EC_{50} were > 315 mg test item/kg dry soil. The NOEC was ≥ 315 mg AMPA/kg dry soil, the highest tested concentration, since AMPA had no negative effects on the test organisms.

The study is considered valid so EC_{50} > 315 mg/kg dry soil and NOEC ≥ 315 mg/kg dry soil can be used in the regulatory risk assessment for *Folsomia* exposed to AMPA.

Assessment and conclusion by RMS:

Test item: AMPA
Test item was mixed into the soil.

The study was conducted according to ISO 11267 (1999) and OECD 232 (2009). The validity criteria of OECD 232 (2016) are fulfilled, and the study design is in line with the recommendations of this current guideline.
Deviations from guideline OECD 232 (2016):
- 30 g wet weight per test vessel was used instead of 30 g dry weight.
RMS considers this is minor deviation.

RMS notes that a detailed description of the extraction efficiency is lacking.
The number of parental and juvenile collembolans (floating on the surface) was determined by counting adults and juveniles by means of a digital image processing system (LemnaTec Scanalyzer). This is an automated counting technique based on a video camera connected with a frame grabber. By scanning the surface of the container with the digital image processing system, the number of juvenile and adult individuals was quantified using the image processing software from LemnaTec. The study author states that the requirement of the former ISO guideline concerning the precision of the counting method (average error <10 %) was fulfilled, and that the determined overall error of counting amounted to 2.5 %.
OECD 232 requires that the validity includes extraction efficiency of juveniles greater than 95%, e.g. by adding a known number to soil. However no further information could be retrieved in this study report.

Reference test:
Reference item boric acid performed well.

This study is considered valid.

28d NOEC = 315 mg AMPA/kg dry soil
EC50 > 315 mg AMPA/ kg dry soil, highest concentration tested.

No EC10 or EC20 have been proposed. However effects being less than 10%, this is considered unnecessary.

Data point:	CA 8.4.2.1/004
Report author	[redacted]
Report year	2010
Report title	AMPA – Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*
Report No	10 10 48 053 S
Document No	-
Guidelines followed in study	OECD 226 (2008)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guideline OECD 226 (2016):* *Minor:* *- A combined approach design (determination of NOEC and EC_{50}) was conducted with only 5 test item concentrations and a spacing factor of 2 (8 concentrations and spacing factor not exceeding 1.8 are required).*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary
In the laboratory study the toxicity and reproductive inhibition of AMPA to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 40, 80, 160, 240 and 320 mg test item/kg dry soil and to deionised water only as control. A toxic reference (Dimethoate EC 400) was tested in a separate study.
40 mites (10/test vessel) per test concentration and 80 mites per control (10/test vessel) were put in a glass bottle on artificial soil with incorporated test item and adults and juveniles counted after 14 days. The test item AMPA caused no statistically significant mortality of adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed.
This study is considered valid.The 14d NOEC is 320 mg AMPA/kg soil d.w. and EC50 > 320 mg AMPA/kg soil d.w., highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solid
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7%

2. Vehicle of test material and positive control:

Vehicle: deionised water

Positive control: Reference item: Dimethoate EC 400 (414.8 g/L analysed)

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini)

Age:	Adult mites
Source:	In-house culture originally obtained from Katz Biotech AG, 15837 Baruth, Germany
Diet/Food:	*Tyrophagus putrescentiae* (Schrank) were fed every 2 days, before and during the test

4. Environmental conditions:

Temperature:	19.7 – 21.8 °C
Composition of artificial soil	5% sphagnum peat 20% kaolin clay 0.3% calcium carbonate 74.7% quartz sand Deionised water
Soil water content:	Test start: 17.40- 18.07% (47.81 – 49.64% of WHC) Test end: 17.10 – 17.55% (46.98 – 48.22% of WHC)
pH	Test start: 5.8 – 6.1 Test end: 5.4 – 6.3
Photoperiod:	16 hours light / 8 hours darkness
Light intensity:	472 lux

B. STUDY DESIGN AND METHODS

1. Experimental treatments: AMPA was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at five test item concentrations of 40, 80, 160, 240 and 320 mg test item/kg dry soil. In addition, a control with deionised water and a toxic reference (Dimethoate EC 400) were conducted. Each test item concentration was tested with 40 mites (10/test vessel), while the control group consisted of 80 mites (10/test vessel). For each test item concentration and for the control group 2 test vessels without mites were provided for measurement purposes. The mites were put in glass bottles with screw tops of 100 mL, each containing 20 g (dry weight) artificial soil with the requested test item concentrations and closed. Every two days test vessels were opened for food supply and aeration. Two weeks after introducing the test organisms the parental and juvenile mites were counted.

2. Observations: Water content and pH were determined at test start and end. Adults and juvenile mites were counted at test end. The temperature was continuously measured and recorded.

3. Statistical calculations: Fisher's Exact Binomial test with Bonferroni Correction for significance of parental mortality. Dunnett-t-test ($p \leq 0.05$) for significance of reproductive reduction. Statistical program: ToxRat Professional 2.10 (2009).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.4.2.1-4: Mortality and reproductive reduction of *Hypoaspis aculeifer* after application of AMPA in a 14 day laboratory study

Test concentration [mg test item/kg dry soil]	Mortality of adults after 14 days [%]	Corrected mortality [1] [%]	Mean number of juveniles after 14 days [%]	SD	Reduction of reproduction compared to control [%]	Coefficient of variation [%]
Control	0.0	-	220.6	29.5	-	13.3
40	5.0	5.0	228.0	47.2	-3.3	20.7
80	2.5	2.5	236.3	18.2	-7.1	7.7
160	2.5	2.5	209.3	12.6	5.2	6.0
240	0.0	0.0	237.3	23.4	-7.5	9.9
320	2.5	2.5	227.5	47.2	-3.1	20.7

[1] calculated with Abbott 1925

Reference test:
After treatment with the reference item Dimethoate EC 400 at concentrations of 4.1, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil and EC_{50} (reproduction) of 6.6 mg test item/kg dry soil was concluded.

B. OBSERVATIONS

The test item AMPA caused no statistically significant mortality (Fishers's Exact Binomial Test, $p > 0.05$) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Also, no significant decrease in reproduction was observed (Dunnett-t-test, $p > 0.05$).

The EC_{50} value and the NOEC are given below based on nominal concentrations.

Endpoints	AMPA [mg/kg dry soil]
NOEC	320
EC_{50} (14 d)	> 320

Reference test:
The EC_{50} (reproduction) with the reference item Dimethoate EC 400 was in line with the range defined in the guideline to demonstrate the sensitivity of the test system.

According to the applicant, the following point deviated from the guideline OECD 226 (2016):
A combined approach design (determination of NOEC and EC_{50}) was conducted with only 5 test item concentrations and a spacing factor of 2 (8 concentrations and spacing factor not exceeding 1.8 are required). Since an EC_{50} could not be calculated and would be greater than the highest test concentration, the design is in line with the requirement for determination of NOEC only.

All validity criteria according to OECD 226 were fulfilled, as adult mortality did not exceed 20%, the mean number of juveniles per replicate was > 50 at test end and the coefficient of variation of the number of juveniles per replicate was not higher than 30% at test end.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The effects of AMPA on mortality and reproduction of *Hypoaspis aculeifer* were assessed for 14 days under laboratory conditions.

The 14-day EC_{50} was > 320 mg test item/kg dry soil. The NOEC was ≥ 320 mg AMPA/kg dry soil, the highest tested concentration, since AMPA had no negative effect on the test organisms.

The study is considered valid so EC_{50} > 320 mg/kg dry soil and NOEC ≥ 320 mg/kg dry soil can be used in risk assessment for *Hypoaspis* exposed to AMPA.

Assessment and conclusion by RMS:

Test item: AMPA
Test item was mixed into the soil.

The study was conducted according to OECD 226 (2008). The validity criteria of OECD 226 (2016) are fulfilled, and the study design is in line with the recommendations of this current guideline.

According to the applicant, the following point deviated from the guideline OECD 226 (2016):
A combined approach design (determination of NOEC and EC_{50}) was conducted with only 5 test item concentrations and a spacing factor of 2 (8 concentrations and spacing factor not exceeding 1.8 are required). Since an EC_{50} could not be calculated and would be greater than the highest test concentration, the design is in line with the requirement for determination of NOEC only. RMS confirms that the design of this study still addresses the requirements for determination of a NOEC.

About extraction efficiency :
The study author states that the extraction efficiency of the extractor was determined to be 94% in a separate extraction run using vessels containing a known number of juveniles and adult mites kept in untreated test substrate (current guideline OECD 226 requires >90%). However, a detailed description of the extraction efficiency is lacking.

Reference test:
The toxic reference performed well.

This study is considered valid.

14d NOEC = 320 mg AMPA/kg soil d.w.
EC50 > 320 mg AMPA/kg soil d.w., highest concentration tested.

Effects being less than 10%, EC10 and EC20 values could not be derived.

Data point:	CA 8.4.2.1/005 , also referenced under CA 8.5/005, also referenced under CA 8.4.1/005
Report author	von Mérey G. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota
Document No	DOI: 10.1002/etc.3438 E-ISSN: 1552-8618
Guidelines followed in study	OECD 222; OECD 226; OECD 232; OECD 216
Deviations from current test guideline	Earthworm cocoons were not counted, in accordance with OECD 222.
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability **(RMS)**:	**Assessment and conclusion by RMS:** This publication actually corresponds to the regulatory studies summarized and already assessed by RMS CA 8.4.1/001 [redacted] 2009 Glyphosate IPA salt Eisenia andrei CA 8.4.1/003 [redacted] 2003 AMPA Eisenia fetida CA 8.4.1/001 [redacted] 2010 Glyphosate IPA salt Folsomia candida CA 8.4.1/002 [redacted] 2009 Glyphosate IPA salt Hypoaspis aculeifer CA 8.4.2.1/003 [redacted] 2010 AMPA Folsomia candida CA 8.4.2.1/004 [redacted] 2010 AMPA Hypoaspis aculeifer CA 8.5/001 [redacted] 2014 Glyphosate Nitrogen transformation CA 8.5/004 [redacted] 2010 AMPA Nitrogen transformation Therefore, this publication was not assessed by RMS.

See under Volume 3 CAB.9, point B.9.4.1

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Data point	CA 8.5/001
Report author	[redacted]
Report year	2014
Report title	Glyphosate technical (MON77973): Effect on Soil Microbial Nitrogen Transformations
Report No	CEMR-6237
Document No	-
Guidelines followed in study	OECD Guideline 216 (2000)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 2016 (2000): None*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

The effects of Glyphosate technical (MON 77973) on the nitrogen transformation pathways were assessed in a LUFA standard soil type 2.3. The transformation rates were determined in replicate soil samples treated with MON 77973 at rates of 6.62 and 33.1 mg acid equivalent/kg dry soil and compared to a control (deionised water). The products of the process of nitrification (nitrate, ammonium and nitrite) were extracted from the soil on Day 0, 7, 14 and 28 after treatment.
As the average rate of production of nitrate (mg/kg/day) from Day 14 to Day 28 between the treatment rates of MON 77973 (6.62 and 33.1 mg a.e./kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on nitrogen transformation in soils.
The study is considered valid and NOEC = 33.1 mg a.e./kg dry soil can be used in risk assessment for micro-organisms exposed to glyphosate technical.

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	Glyphosate technical (MON 77973)
Description:	White Powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	96.59% Glyphosate Acid
Vehicle of test material/media:	Vehicle: deionised water

Test system:

Soil	Sandy loam soil “LUFA standard soil 2.3” (Batch number Sp2.33113)
Source:	LUFA-Speyer, Obere Langgasse 40, 67346 Speyer, Germany
Water holding capacity:	36.2% (g water/100 g dry soil)

Water content:	35 ± 5%
pH:	6.5
Total Org. Carbon:	0.67%
Microbial biomass:	4.35% of TOC
Clay (< 0.002 mm):	5.9 ± 2.5%
Silt (0.002 - 0.050 mm):	33.9 ± 0.0%
Sand (0.050 – 2.0 mm):	60.3 ± 2.5%
Acclimation:	35% (± 5%) of MWHC at 20 ± 2°C for 5 days

Environmental conditions:

Temperature:	20 ± 2°C
pH:	6.0 - 6.6 (range between Day 0 and Day 28)
Water content:	42% of MWHC
Photoperiod:	24 hours darkness
Experimental Dates:	20 September - 24 October 2013

B. STUDY DESIGN AND METHODS

Experimental treatments

Soil samples were bulk dosed with MON 77973 at nominal rates equivalent to 1 and 5 × $PEC_{plateau}$ (6.62 and 33.1 mg a.e./kg dry soil, respectively).

Five days before the start of the exposure phase, the soil moisture content was nominally adjusted to 35% (± 5%) of the MWHC. The soil was placed in the test cabinet in the dark at 20 ± 2 ºC. On the day of dosing, the moisture of the soil was adjusted to 40% (± 5%) of the MWHC with deionised water with the appropriate dose of test item. Three replicates (each of them contained 500 g dry weight equivalent of soil) were prepared for the control treatment (deionised water) and the test item treatments. Each replicate of soil was transferred to plastic test vessels (2 L). The test soil was amended with lucerne (2.5 g of lucerne/500 g of soil) to the control and treatment groups on Day 0. Additionally, 500 g (dry weight equivalent) of soil was prepared which had no lucerne amendment to serve as the unamended control sample. The moisture content of soil samples was maintained during the test at 40% of the maximum water holding capacity of the soil with a range of ± 5%.

Inorganic ammonium, nitrate and nitrite were extracted from each sub-sample of soil with 2 M potassium chloride solution (250 mL) and shaking for 2 hours. The extract was separated from the soil by centrifugation (15 minutes, 2500 rpm). Approximately 20 mL of the supernatant was stored refrigerated prior to analysis. Each extract was analysed for nitrate, ammonium and nitrite using the Bran + Luebbe Autoanalyser AA3 system.

Observations

As soon as possible after treatment, a sub-sample of soil was taken from each replicate for the determination of nitrate, nitrite and ammonium concentration. Further sub-samples were taken after 7, 14 and 28 days. All samples were analysed for nitrate, ammonium and nitrite on Day 28. Concentrations of nitrate (as TON) and ammonium were measured (mg/kg dry soil) from Day 0 to Day 28. The nitrite values were not reported as no nitrite-N was detected, and therefore considered not to have nitrite present in any of the extracted soil solutions. Changes in concentration of nitrate and nitrate transformation rates (mg/kg/day) over the duration of the study were measured. The changes in nitrate production from 0 - 7, 7 - 14 and 14 - 28 days were also determined.

Statistical calculations

Shapiro-Wilks and Bartlett's Test followed by Dunnett's two-tailed test (α= 0.05).

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-1: Effects of MON 77973 on soil nitrogen transformation

		Nitrogen concentration [mg/kg soil]		% deviation from control	
Concentration in MON 77973	**Control**	**6.62 mg/kg dws**	**33.1 mg/kg dws**	**6.62 mg/kg dws**	**33.1 mg/kg dws**
Nitrate transformation rates					
Day 0-7	-3.47	-3.51	-3.56	+1.26	+2.52
Day 7-14	+1.04	+1.34	+1.39	+29.47	+33.68
Day 14-28	+4.10	4.09	+4.18	-0.13	+2.13
Nitrate (NO_3^-)					
Day 0	24.3	24.6	24.9	+1.23	+2.47
Day 7	0	0	0	-	-
Day 14	7.3	9.4	9.7	+28.77	+32.88
Day 28	64.6	66.7	68.3	+3.25	+5.73
Ammonium (NH_4^+)					
Day 0	7.0	7.0	6.6	0	-5.71
Day 7	2.4	2.4	2.4	0	0
Day 14	1.8	1.7	1.7	-5.56	-5.56
Day 28	0.8	0.8	0.8	0	0

dws: dry weight soil
- = inhibition, + = stimulation

B. OBSERVATIONS

Statistical analysis showed there was no significant difference ($p<0.05$) between the treatment rates of 6.62 and 33.1 mg a.e./kg dry soil and the control treatment for nitrate production from Day 14 to 28.

As the difference in nitrate production between the treatment rates of MON 77973 (6.62 and 33.1 mg a.e./kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on nitrogen transformation in soils at concentrations ≤33.1 mg a.e./kg dry soil.

The variation within the control treatment ranged from -4.2 to 2.6% at Day 0; from -0.9 to 1.8% at Day 7; from -49.5 to 26.3% at Day 14 and from -7.1 to 5.4% at Day 28.

The changes in nitrate production were determined between each time point and not on the whole test from 0-28 days.

Validity criteria

The validity criterion according to OECD 216 guideline was met at study termination, as the variation between replicate control treatments did not vary by more than ± 15% at Day 28 for nitrogen transformation (actual values from -7.1 to 5.4%).

III. CONCLUSIONS

Assessment and conclusion by applicant:
The study provides relevant and reliable endpoints to be used in the regulatory risk assessment for Glyphosate. At soil concentrations of 6.62 and 33.1 mg glyphosate acid equivalent/kg dry soil, there were <25% effect at Day 28 in nitrogen transformation, so MON 77973 is expected to have no long-term influence on the nitrogen transformation pathways in soils up to and including a test concentration ≤33.1 mg glyphosate acid equivalent/kg dry soil.

The study is considered valid and NOEC ≥ 33.1 mg a.e./kg dry soil (corresponding to 24.8 kg a.e./ha) can be used in risk assessment for micro-organisms exposed to glyphosate technical.

Assessment and conclusion by RMS:
This study is valid.
It seems that no nitrate was measured at day 7 in none of the treatments including control. The study do not report any malfunction nor comments the absence of nitrate at this time point. Since this happened at an intermediate point, RMS is not concerned since further time points showed normal behaviour. The applicant is requested to provide clarification on this point (data gap).
The conditions of the test and the soil used were adequate.
At soil concentrations of 6.62 and 33.1 mg glyphosate acid equivalent/kg dry soil, there were <25% effect at Day 28 in nitrogen transformation.

Data point:	CA 8.5/002
Report author	[redacted]
Report year	2000
Report title	Side-Effects of Glifosate Técnico [redacted] on soil microflora: Carbon and Nitrogen Cycles
Report No	RF-D1.113/99
Document No	-
Guidelines followed in study	Instituto Brasileiro do Meio ambiente e dos Recursos naturais Renováveis_Ibama, portaria Normativa no 84 of October, 15 1996
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guidelines OECD 216 (2000) and OECD 217 (2000):* *Major:* *- Nitrogen cycle evaluation should have been prolonged until deviation from control dropped under ±25%.* *Minor:* *- Detail of soil storage and pre-incubation period are not reported.* *- The alfalfa amendment was also added in samples used for carbon cycle.* *- Carbon cycle was assessed for one hour instead of 12 consecutive hours.* *- The assessments after 7 days were missing for both nitrogen and carbon cycles.*
Previous evaluation	Not accepted in RAR (2015) for nitrogen Yes, accepted in RAR (2015) for carbon
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Executive Summary

The effects of glyphosate technical on soil carbon cycle and nitrogen cycle were investigated in two soil types, a “Typic Hapludox” and a “Rhodic Hapludox” under laboratory conditions. The test substance was applied at two concentration rates of 2.4 and 4.8 kg test item/ha in three replicates. In addition, negative controls (without test item) with or without organic matter amendment were tested. 150 g soil samples were amended with organic matter at a rate of 0.5% dry soil equivalent for all treatments, except for control without organic matter amendment. Soils were incubated at a temperature range of 19 to 22°C in the dark in covered glass flasks. Soil samples were removed from the jars 0, 14 and 28 days after treatment and analysed for soil dry mass, pH, nitrite, nitrate, ammoniacal nitrogen and short term respiration.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Description:	White powder
Lot/Batch #:	037-919-113

Purity:	95% a.s. (nominal), 95.49% a.s. (measured)
2. Vehicle of test material:	deionised water
3. Test system:	
Soil	LE (Typic Hapludox) and LR (Rhodic Hapludox)
Source:	Not stated
Water content of soil:	Not stated
Water holding capacity	Not stated
pH:	5.5 (LR), 7.0 (LE)
Organic matter:	31 g/md^3 (LR) and 20 g/dm^3
Microbial biomass:	2.63 mg C/g soil (LR), 2.24 mg C/g soil (LE)
Clay (< 0.002 mm):	39% (LR), 24% (LE)
Silt (0.002 mm - 0.063 mm):	10% (LR), 9% (LE)
Sand (0.063 – 2.00 mm):	51% (LR), 67% (LE)
4. Environmental conditions:	
Temperature:	19 - 22°C
pH:	5.53 – 6.27 (LR); 6.34 – 6.84 (LE)
Water content:	40- 60% of WHC
Photoperiod:	24 hours dark

B. STUDY DESIGN AND METHODS

1. Experimental treatments: The test substance was applied at two concentration rates of 2.4 and 4.8 kg test item/ha using three replicates per concentration. In addition, negative controls (without test item) with or without organic matter amendment were tested. 150 g soil samples were amended with organic matter at a rate of 0.5% dry soil equivalent for all treatments, except for control without organic matter amendment. Soils were incubated at a temperature range of 19 to 22°C in the dark in covered glass flasks. Soil samples were removed from the jars, 0, 14 and 28 days after treatment and analysed for soil dry mass, pH, nitrite, nitrate, ammoniacal nitrogen and short term respiration test.

2. Observations:

Nitrogen cycle: For the preparation of soil extract for ammonium-N analysis, 10 g of soil was placed in 250 mL wide-mouth bottle, to which 100 mL of 2M KCl was added. 1 mL of the filtered aliquot containing between 0.5 and 12 µg of NH_4+-N was placed into 25 mL volumetric flasks. 1 mL EDTA, 2 mL phenol nitroprussid and 4 mL hypochlorite buffer were successively added. The concentration of NH_4+-N was thereafter determined using a photometric method at 636 nm. For nitrate-N and nitrite-N analysis, 10 g of soil was placed in a 500 mL Erlenmeyer flask, then 0.5 g of $CaSO_4$ and 250 mL distilled water were added. For the analysis of nitrate-N, an aliquot of 25 mL of the extract was pipetted into 10 mL round bottom flask and 0.05 g of $CaCO_3$ was added. Subsequently, 2 mL of phenoldisulfonic acid (25 g phenol in 150 mL of concentrated H_2SO_4) was added. After 10 min, 20 mL of distilled water was added. The nitrate-N concentration was determined using a Hach Model DR 2010 absorbance spectrophotometer at 410 nm. For the analysis of nitrite-N, an aliquot of 25 mL of the extract was pipetted into a 25 mL cell. The visual absorbance of each sample was determined at 507 nm using a Hach Model DR 2010 absorbance spectrophotometer.

Carbon cycle: 2 g of soil samples were placed in 50 mL Erlenmeyer flasks, adding 0.5 mL of 2 µmol/mL of glucose- ^{14}C. In order to absorb CO_2 evolved from glucose degradation by soil microorganisms, a small glass flask (1 mL) was hung from the rubber cap, containing 0.2 mL of NaOH. After one hour of incubation in dark conditions, the glucose degradation was then stopped. The NaOH and filter paper strips were transferred into scintillation vials. The radioactivity was assessed in a Liquid Scintillation

Analyzer Packard model Tri-carb 1900, during 5 min/sample.

3. Statistical calculations: Results were evaluated using Duncan's Multiple range Test at $\alpha = 0.01$.

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.5-2: Effects of glyphosate technical on soil nitrogen cycle

		Glyphosate technical [kg test item./ha]				
		Control	2.4		4.8	
		[mg N/kg dry soil]	[mg N/kg dry soil]	Dev. [a]	[mg N/kg dry soil]	Dev. [a]
Soil: LR (Rhodic Hapludox)						
Day 0	Ammonium	22.66	21.61	-4.6	24.31	+7.3
	Nitrite	0.30	0.29	-3.3	0.40	+33.3*
	Nitrate	22.51	22.54	+0.1	23.11	+2.7
Day 14	Ammonium	27.34	34.92	+27.7*	37.50	+37.2*
	Nitrite	0.29	0.21	-27.6*	0.23	-20.7
	Nitrate	30.02	36.47	+21.5*	44.10	+46.9*
Day 28	Ammonium	13.13	11.32	-13.8	9.38	-28.6*
	Nitrite	0.26	0.24	-7.7	0.24	-7.7
	Nitrate	18.39	24.16	+31.4*	34.61	+88.2*
Soil: LE (Typic Hapludox)						
Day 0	Ammonium	30.01	27.87	-7.1	34.72	+15.7*
	Nitrite	0.32	0.27	-15.6	0.27	-15.6*
	Nitrate	22.58	22.74	+0.7	23.34	+3.4
Day 14	Ammonium	26.19	22.60	-13.7	24.50	-6.5
	Nitrite	0.26	0.29	+11.5	0.27	+3.8
	Nitrate	21.78	39.26	+80.3*	41.01	+88.3*
Day 28	Ammonium	16.82	18.71	+11.2	18.72	+11.3
	Nitrite	0.40	0.24	-40.0*	0.26	-35.0*
	Nitrate	18.39	31.67	+72.2*	25.77	+40.1*

[a] - = Deviation from control
* = Significant deviation from control according to OECD Guideline 216
- = inhibition, + = stimulation

Table B.9.5-3: Effects of glyphosate technical on soil carbon cycle

	Glyphosate technical [kg test item/ha]				
	Control	**2.4**		**4.8**	
	Soil respiration [b]	**Soil respiration [b]**	**Dev. [a]**	**Soil respiration [b]**	**Dev. [a]**
Soil: LR (Rhodic Hapludox)					
Day 0	9.00	8.33	-7.4	9.06	+0.7
Day 14	16.06	16.19	+0.8	16.76	+4.4
Day 28	15.13	14.63	-3.3	16.53	+9.3
Soil: LE (Typic Hapludox)					
Day 0	12.80	13.00	+1.6	11.56	-9.7
Day 14	16.69	20.16	+20.8	17.56	+5.2
Day 28	16.43	18.06	+9.9	17.26	+5.1

[a] - = Deviation from the control
[b] = Activity of soil microorganism in mmoles metabolized glucose/g soil/h
- = inhibition, + = stimulation

B. OBSERVATIONS

No adverse effects of glyphosate technical on soil carbon cycle were observed for both concentrations 28 days after application. In addition, all validity criteria according to OECD 217 were fulfilled. For the soil nitrogen cycle test validity criteria according to OECD 216 were not fulfilled, as the variation between replicate control samples was more than ± 15%.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The test item glyphosate technical caused no significant adverse effects on soil carbon cycle at test concentrations of 2.4 and 4.8 kg test item/ha, 28 days after treatment.
All validity criteria according to OECD 217 were fulfilled. For the soil nitrogen cycle test however, the validity criteria according to OECD 216 were not fulfilled, as the variation between replicate control samples was more than ± 15%. Therefore, no consistent conclusions could be drawn from the study.
The study is therefore considered invalid.

Assessment and conclusion by RMS:
Nitrogen cycle:
In RAR 2015, the former RMS already highlighted that for soil nitrogen cycle (OECD 216) test the variation between replicate control samples was more than ± 15%. This being a validity criteria, the study is not considered valid for the part related to nitrogen transformation.

Carbon cycle:
This is not a data requirement anymore so it is left for information purpose only.
Several minor deviations are noted by the applicant. RMS also notes that WHC is not reported.
The test item glyphosate caused no adverse effects on soil carbon cycle at test concentration of 2.4 and 4.8 kg a.s/ha, 28 days after treatment.

Data point:	CA 8.5/003
Report author	[redacted]
Report year	1995
Report title	The Effects of Glyfosaat on Soil Respiration and Nitrification
Report No	141885
Document No	-
Guidelines followed in study	BBA-Guideline: Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln Teil VI 1-1 (2. Auflage). "Auswirkungen auf die Aktivität der Bodenmikroflora", (März, 1990)
Deviations from current test guideline identified by the applicant: See RMS analysis in RMS comment box	*Cannot be checked*
Previous evaluation	No
GLP/Officially recognised testing facilities	Cannot be checked
Acceptability/Reliability (RMS)	Invalid

Assessment and conclusion by applicant:
Glyphosate had no significant long term detrimental effect on microbial biomass and nitrogen content in soil at concentrations of 2.88 and 14.4 mg/kg dry soil. It is not possible to conclude on the study validity according to current OECD guideline requirements. The study is therefore considered invalid.

Assessment and conclusion by RMS:
This study was not assessed in RAR 2015.
Only a report amendment was submitted by the applicant (study summary not available). However severe drawbacks were highlighted. RMS also notes that this amendment concludes to the absence of adverse effect of the test item. As a new study is available (for the active substance) that was conducted according to the current guideline, this study is not considered essential by RMS.
The study summary proposed by the notifier was therefore not reported.

Data point	CA 8.5/004
Report author	[redacted]
Report year	2010
Report title	AMPA - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests)
Report No	10 10 48 010 C/N
Document No	-
Guidelines followed in study	OECD 216 (2000) OECD 217 (2000)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from guidelines OECD 216 (2000) and 217 (2000):* *Minor:* *- Deviation from nitrate formation rate is missing.*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid / Reliability for risk assessment to be confirmed

Executive Summary

The effects of AMPA on soil nitrogen transformation and soil carbon transformation were investigated in a loamy sand soil. The test substance was applied at concentration rates of 40, 80, 160, 320 and 640 mg test item/kg dry soil using three replicates per treatment. In addition, a negative control (untreated soil) was tested. A reference item was tested in a separated study.
The results showed no adverse effects of the test item 28 days after application on nitrogen and carbon transformation in soil up to and including a test concentration of 160 mg test item/kg dry soil. Due to measured deviations of > 25% observed in the treatment groups treated with 320 and 640 mg test item/kg dry soil, 28 days after application, the test was prolonged to 56 days for both treatment levels. After the test prolongation, the measured variations of nitrogen and carbon transformations of >25% could be observed until the end of the study (56 days).
All validity criteria according to OECD 216 and 217 were fulfilled.
The applicant considered this study valid so NOEC of 160 mg/kg of dry soil can be used in risk assessment for micro-organisms exposed to AMPA.
However, RMS considered this study as supportive only (see commenting box).

I. MATERIALS AND METHODS

A. MATERIALS

Test material:

Test item:	AMPA (Aminomethylphosphonic acid)
Description:	White crystalline solid
Lot/Batch #:	GLP-0908-19984-A
Purity:	98.7%
Vehicle of test material and positive control:	Vehicle: deionised water Positive control: Dinoterb

Test system:

Soil	Loamy sand soil "Wassergut Canitz" (agricultural soil)
Source:	Field "Schag 34/3" in the municipality of Canitz, Saxony, Germany.
Water content of soil:	11.30% (g water/100 g dry soil)
Water holding capacity	36.56% (g water/100 g dry soil)
pH:	6.3
Total Org. C:	1.43%
Microbial biomass:	2.37% of TOC.
Clay (< 0.002 mm):	9.1%
Silt (≥0.002 mm - 0.063 mm):	40.2%
Sand (≥ 0.063 – 2.00 mm):	50.7%

Environmental conditions:

Temperature:	19.7 – 21.8°C
pH:	5.9 – 6.3
Water content:	41.46 – 44.71% of WHC (nitrogen transformation test) 41.84 – 45.09% of WHC (carbon transformation test)
Photoperiod:	24 hours darkness
Experimental work dates:	20 May to 15 July 2010

B. STUDY DESIGN

Experimental treatments

The test substance was applied at concentration rates encompassing 40, 80, 160, 320 and 640 mg test item/kg dry soil. In addition, a negative control (untreated soil) was tested. Three replicate soil samples were prepared for each treatment rate and the control for the carbon transformation and nitrogen transformation tests.

Soil carbon transformation: For each replicate a sub-sample of 1000 g dry soil was mixed with deionised water. Water was added to the soil to achieve a water content of approximately 45% WHC. Water content was adjusted weekly to the required range of 40-50% of WHC. The prepared soil was transferred to steel test vessels (4 L) and incubation was carried out at 19.7 – 21.8°C in a climatic room.

Soil nitrogen transformation: Sub-samples of 200 g dry soil were weighed into each test vessel (500 mL wide mouth glass flask). Lucerne meal (5 g/kg dry soil) was then added to provide 1.0 g Lucerne meal per 200 g dry soil. One additional soil sample (without Lucerne meal) was used for determination of initial NH_4-N- and NO_3-N-content. The initial NH_4-N and NO_3-N content was 0.01 mg and 1.48 mg/100 g dry soil, respectively. Incubation of the prepared soil was carried out in wide-mouth glass flasks (500 mL) at 19.7 – 21.8°C in a climatic room.

Observations

Soil carbon transformation: Carbon transformation was determined for a measurement period of 12 hours on sampling days 0 (3 hours after application), 7, 14, 28, 42 and 56 days after application. On each sampling occasion, 100 g samples of soil (dry soil) were taken, mixed with glucose using a hand-stirrer and placed into glass reaction flasks (500 mL). Then, glass vessels containing 18 mL of 1 M NaOH solution were placed in the reaction flasks and connected with a respirometer (BSB digi SELUTEC). Cumulative oxygen production (corresponding to the O_2 consumption by micro-organisms) was determined over a 12-hour measurement period.

Soil nitrogen transformation: Soil samples (10 g dry soil per replicate) were sampled at intervals of 3 hours, 7, 14, 28, 42 and 56 days after application and NH_4-N, NO_3-N and NO_2-N contents were

determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g dry soil. Quantitative determination of mineralized nitrogen was performed using an Autoanalyzer II.

Statistical calculations

Two-sided Students t-test for homogenous variances at $\alpha = 0.05$. For carbon transformation, a two-sided Welch t-test for inhomogeneous variance was additionally performed.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

28 days after application, no adverse effects on nitrogen content and carbon transformation were observed up to and including a test concentration of 160 mg test item/kg dry soil. After the prolongation of the test to 56 days for the test concentrations 320 and 640 mg test item/kg dry soil, the measured variations of nitrogen content and carbon transformations of >25% could be observed till the end of the study (56 days). This can be most likely attributed to the high phosphorus/nutrient content in AMPA.

Table B.9.5-4: Effects of AMPA on soil nitrogen transformation

	AMPA [mg test item/kg dry soil]										
	Control	40		80		160		320		640	
	NO_3-N	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]	NO_3-N	Dev. [a]
Day 0	15.7	15.5	-1.1	15.7	0.2	15.4	-1.9	14.9*	-4.9	14.6*	-6.6
Day 7	23.1	23.6	2.5	27.3*	18.5	25.8	11.7	30.5*	32.2	33.5*	45.2
Day 14	32.2	34.6	7.5	37.4*	16.3	35.1*	9.2	42.9*	33.3	43.9*	36.5
Day 28	42.2	46.8*	10.7	47.7*	13	51.0*	20.8	57.4*	35.8	65.0*	53.8
Day 42	55.4	-	-	-	-	-	-	72.1*	30.2	78.1*	41.1
Day 56	61.9	-	-	-	-	-	-	78.4*	26.7	88.6*	43.1

[a] - = Deviation from the control based on NO_3-nitrogen content

* = Significantly different from control (two-sided Student- t test for homogenous variances at $\alpha = 0.05$)

- = inhibition, + = stimulation

Table B.9.5-5: Effects of AMPA on soil carbon transformation

	AMPA [mg test item/kg dry soil]										
	Control	40		80		160		320		640	
	O_2 [a]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]	O_2 [a]	Dev. [b]
Day 0	12.0	11.9	-0.8	11.4*	-5.3	11.1*	-8.0	10.8*	-10.4	10.1*	-16.2
Day 7	11.9	11.0*	-7.1	10.3*	-13.2	9.9*	-16.9	9.5*	-20.2	8.4*	-29.7
Day 14	11.7	10.9*	-7.0	10.6*	-9.1	9.9*	-15.4	9.1*	-22.6	8.0*	-31.3
Day 28	10.9	10.0*	-7.9	9.5*	-12.9	8.9*	-18.5	8.1*	-25.7	7.0*	-35.3
Day 42	10.7	-	-	-	-	-	-	7.9*	-26.6	6.8*	-37.0
Day 56	10.1	-	-	-	-	-	-	7.4*	-26.1	6.2*	-38.8

[a] - = Oxygen consumption

[b] - = Deviation from the control

* = Significantly different from control (two-sided Student- t test or two-sided Welch-t-test, for homogenous or inhomogeneous variances at $\alpha = 0.05$, respectively)

- = inhibition, + = stimulation

In a different test, 28 days after application the toxic standard dinoterb caused effects of +37.6%, +51.4% and +27.1% on nitrogen content and -30.5%, -34.5% and -28.8% on carbon transformation at concentrations of 6.80, 16.0 and 27.0 mg dinoterb/kg dry soil respectively, and thus demonstrates the sensitivity of the test system.

All validity criteria according to OECD 216 and 217 were fulfilled, as the variation between replicate control samples was less than ± 15%.

III. CONCLUSIONS

Assessment and conclusion by applicant:
The test item AMPA caused no adverse effects on soil nitrogen content and on soil carbon transformation up to and including a test concentration of 160 mg test item/kg dry soil at the end of the 28-day incubation period.
The study is considered valid so NOEC of 160 mg/kg of dry soil (corresponding to 120 kg/ha) can be used in risk assessment for micro-organisms exposed to AMPA.

Assessment and conclusion by RMS:
This study is valid but the results are supportive only.
AMPA caused no adverse effects on soil nitrogen transformation and on soil carbon transformation (< 25 % deviation from control) up and including a test concentration of 160 mg AMPA/kg dry soil at the end of 28-day period. However this results is considered supportive only. Indeed, the soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day have not been calculated for control and all tested concentrations in order to determine the difference in transformation rates as recommended by the OECD 216. Therefore, RMS considers there is a data gap.

Data gap: applicant to submit soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day

Data point:	CA 8.5/005, also referenced under CA 8.4.2.1/005
Report author	von Mérey, G. *et al.*
Report year	2016
Report title	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota
Document No	DOI: 10.1002/etc.3438 E-ISSN: 1552-8618
Guidelines followed in study	OECD 222; OECD 226; OECD 232; OECD 216
Deviations from current test guideline	Earthworm cocoons were not counted, in accordance with OECD 222.
GLP/Officially recognised testing facilities	No, not applicable
Acceptability/Reliability (RMS):	**<u>Assessment and conclusion by RMS</u>:** This publication actually corresponds to the regulatory studies summarized and already assessed by RMS CA 8.4.1/001 [redacted] 2009 Glyphosate IPA salt Eisenia andrei CA 8.4.1/003 [redacted] 2003 AMPA Eisenia fetida CA 8.4.1/001 [redacted] 2010 Glyphosate IPA salt Folsomia candida CA 8.4.1/002 [redacted] 2009 Glyphosate IPA salt Hypoaspis aculeifer CA 8.4.2.1/003 [redacted] 2010 AMPA Folsomia candida CA 8.4.2.1/004 [redacted] 2010 AMPA Hypoaspis aculeifer CA 8.5/001 [redacted] 2014 Glyphosate Nitrogen transformation CA 8.5/004 [redacted] 2010 AMPA Nitrogen transformation Therefore, this publication was not assessed by RMS.

See under Volume 3 CA B.9, point B.9.4.1

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

Studies on the effects of the active substance glyphosate on vegetative vigour and seedling emergence of terrestrial non-target plants are available and are presented.

B.9.6.1. Summary of screening data

Screening data with the active substance is not considered to be required.
Data on effects of glyphosate on vegetative vigor are available and summarised below. Data are also available for the representative product MON 52276 and summarised under Volume 3 CP B.9.11.

B.9.6.2. Testing on non-target plants

Data point:	CA 8.6.2/001
Report author	[redacted]
Report year	1994
Report title	Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using Glyphosate
Report No	93235
Document No	-
Guidelines followed in study	EPA Guidelines, Subdivision J, Series 123-1 (b)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from test guideline OECD 227 (2006):* *Minor:* *- Five plant per 4 inches pot instead of one or two for bigger plants as corn, soybean, tomato, cucumber.* *- No reference substance or historical data were mentioned in the report.* *- Temperature rose above 22±10°C, the light period was less than 16h per day and the hygrometry dropped under 70±25%.*
Previous evaluation	Yes, accepted in the RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

A vegetative vigour study was conducted exposing six dicotyledonous (soybean, lettuce, cabbage, cucumber, radish and tomato) and four monocotyledonous (oat, ryegrass, corn and onion) plant species to seven nominal test concentrations of glyphosate, encompassing 0.0785, 0.1569, 0.3138, 0.6276, 1.2329, 2.5778 and 5.0436 kg a.e./ha. In addition, one negative control group (treated with deionized water) was tested. Each test concentration was applied in four replicates containing five plants each. In addition radish and tomato were tested using five further nominal concentrations of 0.0049, 0.0099, 0.0202, 0.0392 and 0.0785 kg a.e./ha. Plant height was recorded prior to treatment and 21 days after treatment. Phytotoxicity ratings were recorded 7, 14, and 21 days after treatment. 21 days after treatment, plant material was dried at approximately 100°C for a minimum of 48 hours and dry weight was recorded.

Result showed significant effects of glyphosate treatments on visual phytotoxicity, plant height and plant dry weight in all crops. Except for soybean and onion, glyphosate treatments significantly affected plant survival of all species tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Glyphosate (N-phosphonomethylglycine)
Description:	White powder
Lot/Batch #:	RUD-9302-4778-T (technical) RUD-9203-3961-A (analytical standard)
Purity:	96.6% (technical) 99.8% (analytical standard)

2. Vehicle of test material: Vehicle: deionised water

3. Test organism:

Species: sources	6 Dicotyledons: - soybean: Azlin Seed Co. - lettuce: Germain's Seed Co. - cabbage, radish and tomato: Burpee Seed Co. - cucumber: Carolina Seed Co. 4 Monocotyledons: - corn and onion: Burpee Seed Co. - cucumber: Carolina Seed Co. - oat: Northrup King - Ryegrass: Omni Seed Co

4. Environmental conditions:

Temperature:	19°C – 44°C (base test) 17°C – 40 (test continuation)
Relative humidity:	40% - 90% (base test) 37% - 90% (test continuation)
Photoperiod:	Approx. 14 h light/ 10 h dark at 38212 – 45639 Lux (base test) Approx. 13 h light/ 11 h dark at 24542 – 19052 Lux (test continuation)
Soil pH:	7.9
Soil organic matter content:	1.1%

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Prior to treatment, seedlings were grown in plastic pots (approx.10 cm x 10 cm x 7.6 cm) completely filled with soil/perlite mixture. Soybean, cucumber, oat and corn were planted at a depth of 2.5 cm while the remaining six crops were planted at a depth of 1.3 cm. Each treatment/crop combination was replicated four times. Prior to treatment, seedlings were grown to 1-3 true leaves and then thinned to five plants of uniform height per pot. The plants were treated with seven nominal concentrations, encompassing 0.0785, 0.1569, 0.3138, 0.6277, 1.2329, 2.5780 and 5.0438 kg a.e./ha. In addition, one negative control group (treated with deionized water) was tested. All applications of glyphosate were performed indoors with a spray booth equipped with a single TeeJet 8001-E nozzle and a compressed air cylinder. After treatment plants were placed in greenhouse. During the first 48 hours after treatment, pots were hand watered to prevent the test item from being washed off. As a no-observable effect concentration level was not reached for radish and tomato, a test continuation was initiated for both species using five nominal concentrations, encompassing 0.0049,

0.0099, 0.0202, 0.0392 and 0.0785 kg a.e./ha and a control.

2. Observations: Plant height was recorded prior to treatment and 21 days after treatment. Phytotoxicity ratings were recorded 7, 14, and 21 days after treatment. 21 days after treatment, surviving plants were cut at soil level and dry weight was recorded. Prior to application, samples (10 mL) of each test solution were collected and analysed immediately by HPLC method to verify the concentrations of the test item in the test solutions.

3. Statistical calculations: Analysis of variance, followed by a one-tailed Dunnett's multiple comparison test were used for data analysis. The ER_x values were determined using regression analysis (TableCurve™ Curve Fitting Software).

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

Visual phytotoxicity, plant height and plant dry weight of all crops were significantly affected by glyphosate treatments.

Table B.9.6.2-1: Effects of glyphosate on survival, plant height and plant dry weight at 21DAT (all species, test 1)

	Glyphosate [kg a.e./ha]						
	0.0785	**0.1569**	**0.3138**	**0.6277**	**1.2329**	**2.5780**	**5.0438**
Mean effect on plant survival [% deviation from control]							
Soybean	0	0	0	0	0	0	-15
Lettuce	0	0	0	0	0	-60*	-95*
Radish	0	0	0	-20*	-70*	-100*	-100*
Tomato	0	0	0	-55*	-100*	-100*	-100*
Cucumber	0	0	0	0	0	-20	-75*
Cabbage	0	0	0	0	0	-15*	-60*
Oat	0	0	0	0	-5	-15	-25*
Ryegrass	0	0	0	0	-5	-25*	-50*
Corn	0	0	0	0	-25*	-85*	-70*
Onion	0	0	0	0	0	0	0
Mean effect on plant height [% deviation from control]							
Soybean	0	-7	-3	-10	-52*	-69*	-80*
Lettuce	9	-1	-1	-7	-50*	-86*	-99*
Radish	-11	-16*	-41*	-68*	-89*	-100*	-100*
Tomato	-9*	-11*	-32*	-88*	-100*	-100*	-100*
Cucumber	2	4	-12	-38*	-44*	-66*	-91*
Cabbage	-7	-5	-14	-10	-52*	-74*	-91*
Oat	0	-6	-8	-16	-46*	-77*	-82*
Ryegrass	4	1	5	-1	-22*	-68*	-80*
Corn	-2	-4	-7	-14	-79*	-97*	-92*
Onion	-2	0	-8	0	-27*	-40*	-48*
Mean effect on plant dry weight [% deviation from control]							
Soybean	4	-5	-10	-32*	-66*	-82*	-92*
Lettuce	12	7	-4	-35*	-83*	-97*	-100*
Radish	-25*	-24*	-63*	-85*	-96*	-100*	-100*
Tomato	-11*	-37*	-69*	-98*	-100*	-100*	-100*
Cucumber	6	1	-11	-39*	-63*	-85*	-96*
Cabbage	-5	-3	-24*	-43*	-87*	-96*	-98*
Oat	-3	-2	-17*	-29*	-66*	-92*	-94*
Ryegrass	39	50	27	3	-38*	-91*	-97*
Corn	2	5	-14	-23	-91*	-99*	-98*
Onion	4	15	-10	11	-41*	-71*	-83*

* = Significantly different from the control ($p < 0.05$)

Table B.9.6.2-2: Effects of glyphosate on survival, plant height and plant dry weight (test 2)

	Glyphosate [kg a.e./ha]				
	0.0049	**0.0099**	**0.0202**	**0.0392**	**0.0785**
Mean effect on plant survival [% deviation from control]					
Radish	0	0	0	0	0
Tomato	0	0	0	0	0
Mean effect on plant height [% deviation from control]					
Radish	-3	0	3	-2	-3
Tomato	5	-2	7	0	2
Mean effect on plant dry weight [% deviation from control]					
Radish	15	13	7	4	-9
Tomato	54	33	33	34	5

Analytical results: The average recovery of glyphosate in test media ranged from 100% to 107% and 105% to 110% of the nominal test concentrations for the first test and the test continuation, respectively. As the mean measured content of the test item always ranged between 80 and 120% of nominal in both tests, ecotoxicological endpoints were evaluated using nominal concentrations of the test item.

Except for soybean and onion, a significant effect on mortality was observed for all species exposed to glyphosate. The resulting ER_{50} and NOER values are presented in the table below.

Table B.9.6.2-3: Toxicity of glyphosate to monocotyledonous and dicotyledonous plants

Crop	**Endpoint [kg a.e./ha] (21 days)**			
	Phytotoxicity	**Percentage survival**		
	NOER	**ER_{25}**	**NOER**	**ER_{50}**
Ryegrass	0.6277	2.578	1.2329	4.5955
Corn	0.0785	0.8855	0.6277	1.6813
Onion	0.6277	> 5.0438	5.0438	> 5.0438
Soybean	0.3138	> 5.0438	5.0438	> 5.0438
Lettuce	0.3138	1.5692	1.2329	2.8021
Cucumber	0.1569	2.9142	2.5780	4.0351
Cabbage	0.6277	3.2505	1.2329	4.5955
Oat	0.6277	4.9318	2.5780	> 5.0438
Radish	0.1569	0.4932	0.3138	0.9191
Tomato	0.0785	0.2914	0.3138	0.5156

Table B.9.6.2-3: Toxicity of glyphosate to monocotyledonous and dicotyledonous plants (continued)

Crop	Endpoint [kg a.e./ha] (21 days)					
	Plant height			Dry weight		
	NOER	ER_{25}	ER_{50}	NOER	ER_{25}	ER_{50}
Ryegrass	0.6277	1.0760	2.3538	0.6277	0.8967	1.3450
Corn	0.6277	0.4708	0.9191	0.6277	0.4147	0.7510
Onion	0.6277	1.3450	> 5.0438	0.6277	0.9527	1.7934
Soybean	0.6277	0.6389	1.5692	0.3138	0.4708	0.9751
Lettuce	0.6277	0.7173	1.3450	0.3138	0.4483	0.7622
Cucumber	0.3128	0.5160	1.4571	0.3138	0.4596	0.8967
Cabbage	0.6277	0.7510	1.4571	0.1569	0.3363	0.7398
Oat	0.6277	0.6164	1.3450	0.1569	0.4259	0.8743
Radish	0.0785	0.1569	0.3587	0.0392	0.1569	0.2466
Tomato	0.0392	0.2242	0.3363	0.0392	0.1009	0.1457

The validity criteria according to the OECD 227 were fulfilled. The seedling emergence was at least 70% (actual values from 80 to 99%). In the control, the plants did not exhibit visible phytotoxic effects; the mean plant survival is at least 90 % for the duration of the study (actual value 100%); environmental conditions for a particular species were identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The lowest (worst case) 21 day EC_{50} values of glyphosate were observed for tomato plants and were calculated to be 0.5156, 0.3363 and 0.1457 kg a.e./ha for survival, plant height and dry weight, respectively. The lowest 21-day NOEC values were determined to be 0.0785 kg a.e./ha (tomato and corn), 0.3138 kg a.e./ha (tomato and radish), 0.0392 kg a.e./ha (tomato and radish), and 0.0392 kg a.e./ha (tomato) respectively for visual phytotoxicity, survival, dry weight and plant height.

The study is considered valid so EC_{50} of 146 g a.e./ha and a NOEC of 78.5 g a.e./ha can be used in risk assessment.

Assessment and conclusion by RMS:

Test item: glyphosate

The following deviations were listed by the applicant:

- *Five plant per 4 inches pot instead of one or two for bigger plants as corn, soybean, tomato, cucumber.*

Prior to treatment, seedlings were grown to the 1-3 true leaf stage, then thinned to five plants of uniform height per pot. RMS notes that ideally, after thinning, one single plant should remain for these species to avoid overcrowding and shading of plants by each other for the duration of the test. As an example OECD 227 recommends one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15 cm container. This should avoid crowding of the plants that could affect growth and overlapping of leaves that could affect exposure. Besides, pots were small (approx.10 cm x 10 cm). However, RMS notes that plants were treated at earlier stage (1-3 leaves) instead of 2-4 leaves (OECD 227). RMS assumes that crowding was then limited. Overall RMS considers this deviation acceptable.

- *No reference substance or historical data were mentioned in the report.*
RMS agrees with the applicant that this deviation is acceptable.

- *Temperature rose above 22±10°C, the light period was less than 16h per day and the hygrometry dropped under 70±25%.*
RMS agrees with the applicant that this deviation is acceptable.

The validity criteria according to the current guideline OECD 227 are fulfilled. This study is considered valid.

Phytotoxicity ratings were recorded in the study. The study report only reported graphical representation (histogram) of the frequency of phytotoxicity ratings. It does not allow RMS to propose ECx calculations for phytotoxicity. RMS noted that NOER of tomato, the most sensitive species in this test, was set at 0.0785 for phytotoxicity and 0.0392 kg a.s./ha for dry weight and plant height. This suggest that dry weight and plant height are the most sensitive parameters. However, as there is only a factor 2 on the NOER, RMS proposed a data gap for the applicant to provide ECx for phytotoxicity. The ECx endpoints provided below are provisional.

The lowest 21 day ER50 value of glyphosate acid was observed for tomato plants and was of 0.1457 kg a.s./ha for plant dry weight. (provisional, data gap for ECx values on phytotoxicity)
The lowest 21- day NOER value was determined to be 0.0392 kg a.s./ha (dry weight for tomato and radish and plant height for tomato).

Data point:	CA 8.6.2/002
Report author	[redacted]
Report year	1994
Report title	LX1146-02 (Glyphosate techn.) Tier II Non-Target plant hazard evaluation – Terrestrial vegetative vigor
Report No	14625B018
Document No	236 GLY
Guidelines followed in study	EPA Guidelines, Subdivision J, Series 123-1 (b)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviations from the test guideline OECD 227 (2006):* *Major:* *- No data on seedling emergence were reported.* *- No analytical verification was performed.* *Minor:* *- Five plant per 6 inch pot instead of one or two for bigger plants as corn, soybean, tomato, cucumber.* *- Phytotoxicity and mortality at 21 DAT were missing for initial test.* *- No reference substance or historical data are mentioned in the report.* *- Temperature rose above and below 22±10°C and light period was under 16h per day.*
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS):	Invalid

Executive Summary

A vegetative vigour study was conducted exposing six dicotyledonous (carrot, cucumber, radish, soybean, sunflower, tomato) and four monocotyledonous (field corn, oat, onion, wheat) plant species to five nominal test concentrations of glyphosate, encompassing 0.0056, 0.0112, 0.0235, 0.0471 and 0.0930 kg a.e./ha in four replicates per treatment. In addition, a negative control group treated with deionized water was tested. The application was performed using a single nozzle hand-held, CO_2 pressurized sprayer. Because of poor rate response in most crops, five additional treatment rates were included, encompassing 0.0930, 0.1861, 0.3721, 0.5582 and 0.7442 kg a.e./ha.

Seedling number and plant height were recorded 7 days before treatment (6 DBT for the continuation test), on the day of treatment, 14 days after treatment (13 DAT for the continuation test) and 28 DAT (21 DAT for the continuation test). For the dry weight measurements, plants within a treated replicate were harvested 21 or 28 DAT and dried for a minimum of 24 h at approximately 100°C. Plant survival observations and phytotoxicity were recorded at 7, 14 and 28 DAT for initial test and 6, 13 and 21 DAT for the continuation test.

Plant height, plant dry weight and survival were significantly affected by glyphosate treatments in all species tested. Among monocotyledonous species, oat was most tolerant to glyphosate, while all other species exhibited approximately the same level of sensitivity to glyphosate. Among dicotyledonous species, sunflower and radish were most sensitive for glyphosate, whilst tomato, carrot and soybean showed a moderate sensitivity to glyphosate. Cucumber was the most tolerant species to glyphosate. For phytotoxicity, monocots and dicots were also affected by glyphosate treatments.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item::	Glyphosate technical
Description:	Solid, white
Lot/Batch #:	206-JAK-119-1
Purity:	98.5% (technical)

2. Vehicle of test material: Vehicle: deionised water

3. Test organism:

Source: species	6 Dicotyledons: - Burpee Seed, Warmister, PA: carrot, cucumber, radish, tomato - Farmers supply, Co., Valdosta, GA: sunflower - Pineland Plantation, Newton; GA: soybean 4 Monocotyledons: - Burpee Seed, Warmister, PA: onion, oat - Farmers supply, Co., Valdosta, GA: field corn, wheat

4. Environmental conditions:

Temperature:	Approx. 11.7°C – 37.8°C
Relative humidity:	70% - 94%
Photoperiod:	10 h light / 14 h dark , 43-336 Wm^{-2} (approx. 3071– 24000 Lux for sunlight)
Soil pH:	5.5 - 5.6
Soil organic matter content:	0.94 - 1.5%

B. STUDY DESIGN AND METHODS

1. Experimental treatments: Prior to treatment, seedlings were grown in plastic pots (approx.15 cm round) containing approximately 1 kg of pasteurised sandy soil. Small seeds (carrot, onion, radish and tomato) were planted at a depth of 0.5 to 1 cm and large seeds (field corn, wheat, oat, cucumber, sunflower and soybean) were planted at a depth of 1 to 1.5 cm. Soybean seeds were inoculated with commercial *Rhizobium japonicum*. Four replicate pots for each treatment were prepared for each species tested. At least 7 days prior to application, seedlings were grown to 1-3 true leaves and then thinned to five plants per replicate and their height recorded. The plants were treated with 5 nominal concentrations (adjusted to test item purity), encompassing 0.0056, 0.0112, 0.0235, 0.0471 and 0.0930 kg a.e./ha. In addition, one negative control group (treated with deionized water) was tested. Application was performed using a single nozzle hand-held CO_2 pressurized sprayer, starting with the water control. Plants were not watered during the first 24-hour period to avoid wetting the plants foliage and dislodging spray residue. Because of poor rate response in most crops, a test continuation was initiated at five additional concentration rates, encompassing 0.0930, 0.1861, 0.3721, 0.5582 and 0.7442 kg a.e./ha.

2. Observations: Plant height were recorded 6 or 7 days before treatment (DBT), on the day of treatment, 13 or 14 days after treatment (DAT) and 21 or 28 DAT. For dry weight measurements, plants were harvested 21 or 28 DAT and dried for a minimum of 24 h at approximately 100°C. Plant survival observations were recorded 7 DAT (6 DAT for the continuation test), 14 DAT (13 DAT for the continuation test) and 28 DAT (21 DAT for the continuation test). Phytotoxicity was evaluated 7, 14 and 21 DAT for initial test and 6, 13 and 21 DAT for the continuation test.

3. Statistical calculations: Data were analysed using two-way ANOVA and an LSD test was performed

as post-hoc. The actual EC_x values were estimated by regression analysis using Lotus 1,2,3 Software.

II. RESULTS AND DISCUSSION

A. FINDINGS and OBSERVATIONS

Plant height, dry weight and survival: Height, dry weight and survival of plants were significantly affected by glyphosate treatments in all species tested. Among the monocotyledonous species, oat was most tolerant to glyphosate while all other species exhibited approximately the same level of sensitivity to glyphosate. Among the dicotyledonous species, sunflower and radish were the most sensitive species, whilst tomato, carrot and soybean exhibited moderate sensitivity to glyphosate. Cucumber was the most tolerant species to glyphosate.

Visual phytotoxicity: Visual phytotoxicity was generally expressed within 13 days after the treatment and did not substantially increase by 21 days. Onion exhibited tip burn (necrosis at the leaf tip and margins) at 0.7442 kg a.e./ha but no visual phytotoxicity at any of the lower rates. Oat exhibited visual phytotoxicity at a rate of 0.3721 kg a.e./ha, whereas wheat and field corn showed signs of visual phytotoxicity at rates as lower as 0.1861 kg a.e./ha. For phytotoxicity, onion was the most tolerant monocot while other monocots tested showed approximately the same level of sensitivity to glyphosate. Glyphosate caused multiple shoots to develop at the soil line; higher application rates caused necrosis at the leaf tips. Despite the levels of visual injury observed on field corn, wheat and oat for all concentration tested, the plant height and dry weight were not significantly affected by glyphosate treatments.

For dicots, visual phytotoxicity occurred within 13 DAT and did no increase significantly by 21 days.

Table B.9.6.2-5: Effects of glyphosate on height, dry weight and survival of non-target plants at 21 DAT (test continuation, all species)

Crop	Glyphosate [kg a.e./ha]				
	0.0930	**0.1861**	**0.3721**	**0.5582**	**0.7442**
Mean plant height [% deviation from control]					
Onion	-20.34*	-20.67*	-13.03*	-10.67*	-32.53*
Field corn	2.50*	-15.48*	-15.94*	-28.17*	-44.76*
Oat	6.50	13.93	9.68	1.72	0.27
Wheat	-4.77*	-22.43*	-22.98*	-23.77*	-37.89*
Soybean	5.41	-5.41*	-35.33*	-48.36*	-49.72*
Radish	-14.64*	-33.67*	-23.16*	-100.00*	-100.00*
Cucumber	5.66	-7.03*	-27.96*	-28.53*	-32.86*
Sunflower	25.92*	-47.28*	-62.93*	-100.00*	-100.00*
Tomato	-1.49*	-17.54*	-28.73*	-30.60*	-43.28*
Carrot	0.48	-12.28*	-22.66*	-35.34*	-40.62*
Mean plant dry weight [% deviation from control]					
Onion	-39.06	-50.00	-12.50	3.13	-34.38
Field corn	-5.83*	-24.27*	-33.01*	-45.63*	-53.88*
Oat	5.77	-9.62	-13.46	-20.19	-11.06*
Wheat	-18.33*	-34.58*	-50.00*	-45.28*	-45.14*
Soybean	-8.90	-10.99*	-33.51*	-46.86*	-49.21*
Radish	-29.07*	-54.46*	-57.36*	-100.00*	-100.00*
Cucumber	12.60	13.39	-11.81	20.73	10.43
Sunflower	0.00	-50.22*	-57.24*	-100.00*	-100.00*
Tomato	-18.10	-11.21*	-44.83*	-55.17*	-62.93*
Carrot	13.04	33.70	30.43*	46.74*	50.72*
Mean plant survival [% deviation from control]					
Onion	-5.00	0.00	0.00	-5.00	-5.00
Field corn	0.00	0.00	0.00	0.00	0.00
Oat	0.00	0.00	0.00	-5.00	-5.00
Wheat	0.00	-5.00	0.00	-15.00	-20.00
Soybean	0.00	0.00	0.00	-5.00	0.00
Radish	0.00	-40.00*	-80.00*	-100.00*	-100.00*
Cucumber	0.00	0.00	-10.00*	-40.00*	-20.00*
Sunflower	0.00	-25.00*	-55.00*	-100.00*	-100.00*
Tomato	0.00	0.00	0.00	0.00	0.00
Carrot	5.26	0.00	5.26	-5.26	-5.26

* = significantly different when compared to the control ($\alpha = 0.05$)

Table B.9.6.2-6: Effects of glyphosate on plant height and dry weigh and survival 21 DAT (initial test, onion and radish)

Crop	Glyphosate [kg a.e./ha]				
	0.0056	**0.0112**	**0.0235**	**0.0471**	**0.0930**
Mean plant height [% deviation from control]					
Onion	-2.68	-10.92	-15.52	-11.30	-20.31*
Mean plant dry weight [% deviation from control]					
Onion	-19.23	-26.92	-19.23	-13.46	-28.85
Radish	-33.33	-20.99	-23.46	33.33	-4.94*

* = significantly different when compared to the control ($\alpha = 0.05$)

When comparing the 21-day data, carrot was the most tolerant dicot with a NOER of 0.3721 kg a.e./ha and exhibited no phytotoxicity at rates up to 0.3721 kg a.e./ha. The only injury observed from the glyphosate was slight chlorosis and stunting for carrot. With the exception of soybean (NOER = 0.1861 kg a.e./ha), the NOER for dicots was 0.0930 kg a.e./ha. For radish and sunflower, mortality was observed at the two highest rates tested and significant treatment effects were also noted in plant height and dry weight.
The resulting ER_{50} and NOER values are presented in the table below.

Table B.9.6.2-7: Toxicity of glyphosate to monocotyledonous and dicotyledonous pants

Crop	Endpoint [kg a.e./ha]		
	Survival		
	NOER	**ER_{25}**	**ER_{50}**
Onion	0.7442	> 0.7442	> 0.7442
Field corn	0.7442	> 0.7442	> 0.7442
Oat	0.7442	> 0.7442	> 0.7442
Wheat	0.7442	> 0.7442	> 0.7442
Soybean	0.7442	0.7442	> 0.7442
Radish	0.0930	0.1412	**0.2488**
Cucumber	0.3721	0.6277	> 0.7442
Sunflower	0.1861	0.1939	0.3508
Tomato	0.7442	> 0.7442	> 0.7442
Carrot	0.7442	> 0.7442	> 0.7442

n.d. = not determined

Table B.9.6.2-8: Toxicity of glyphosate to monocotyledonous and dicotyledonous pants

Crop	Endpoint [kg a.e./ha]					
	Dry weight			Plant height		
	NOER	ER_{25}	ER_{50}	NOER	ER_{25}	ER_{50}
Onion	0.0930	n.d.	n.d.	0.0930	0.7442	> 0.7442
Field corn	0.0930	0.297	0.6400	0.0930	0.4607	> 0.7442
Oat	0.7442	> 0.7442	>0.7442	0.7442	> 0.7442	>0.7442
Wheat	0.0930	0.195	0.6478	0.0930	0.4696	> 0.7442
Soybean	0.1861	0.3262	0.6759	0.1861	0.3587	0.6591
Radish	0.0930	0.0942	**0.2623**	0.0930	0.2802	0.6904
Cucumber	0.7442	> 0.7442	> 0.7442	0.1861	0.51	> 0.7442
Sunflower	0.0930	0.1524	0.2959	0.0930	0.1816	**0.2993**
Tomato	0.1861	0.2443	0.5335	0.0930	0.4069	> 0.7442
Carrot	0.3721	0.3284	0.6512	0.1861	0.4349	> 0.7442

n.d. = not determined

The validity criteria according to the OECD 227 were fulfilled, except the fact that no data on seedling emergence in control group were reported.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The lowest (worst case) 21 day EC_{50} values of glyphosate were determined for radish and were calculated to be 0.2488 and 0.2623 kg a.e./ha for survival and dry weight, respectively.
The lowest (worst case) 21 day EC_{50} value of glyphosate was determined for sunflower and was calculated to be 0.2993 kg a.e./ha for plant height.
The lowest 21-day NOEC value was observed for plant height and visual phytotoxicity and determined to be 0.0930 kg a.e./ha. Not all of the validity criteria according to the OECD 227 were fulfilled, because no data on seedling emergence in control group were reported. Due to these limitations, the study is therefore considered invalid for risk assessment purposes.

Assessment and conclusion by RMS:

Test item: glyphosate

Prior to treatment, seedlings were grown to the 1-3 true leaf stage, then thinned to five plants of uniform height per pot. RMS notes that ideally, after thinning, one single plant should remain for these species to avoid overcrowding and shading of plants by each other for the duration of the test. As an example OECD 227 recommends one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15 cm container. This should avoid crowding of the plants that could affect growth and overlapping of leaves that could affect exposure. However, RMS notes that plants were treated at earlier stage (1-3 leaves) instead of 2-4 leaves (OECD 227). RMS assumes that crowding was then limited. Overall RMS considers this deviation acceptable.

The validity criteria according to the OECD 227 (the seedling emergence is at least 70 %) cannot be validated, as no data on seedling emergence in control group were reported (this drawback was already highlighted in RAR 2015).

The study is therefore not acceptable.

Invalid.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Please refer to Appendix to Volume 3 CP B.9 on literature data on biodiversity.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Data point:	CA 8.8/001
Report author	[redacted]
Report year	2000
Report title	Glyphosate technical: Determination of toxicity to *Pseudomonas putida*
Report No	AH0149/A
Document No	-
Guidelines followed in study	Water quality - *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test) International Standard ISO 10712: 1995.
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the current ISO guideline:* *Major:* *- The control inoculum multiplication factor cannot be evaluated (at least 60 is required within the test period)* *Minor:* *- Only two replicates were setup for each test item dilution instead of three.* *- The test solutions were maintained at 27±0.5°C instead of 23±1°C.*
Previous evaluation	Not accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Invalid

Executive Summary

The effects of glyphosate on *Pseudomonas putida* growth inhibition were evaluated in a 16-hour static toxicity test. The test concentrations of 1.0, 3.2, 10, 32, and 100 mg/L in test medium were prepared in duplicate and sterile conditions in conical flasks. Flasks containing 1.0, 3.2, 10, 32, and 100 mg/L (single replicates) of the reference toxic substance (3,5-dichlorophenol) and three control flasks were also prepared. Four mL growth medium, 1 mL inoculum and deionised water were added to obtain a final volume of 50 mL test solution. After shaking at 27.0±0.5°C (in an incubator) for 16±1 hours the optical density of the contents of each flask were measured with a spectrophotometer.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Aspect:	White solid
Lot/Batch #:	R061837 P30
Purity:	97.6%

2. Vehicle of test material and positive control: Vehicle: deionised water / growth medium
Positive control: 3,5-dichlorophenol (97%)

3. Test organism:

Species:	*Pseudomonas putida*, strain NCIMB9494
Source of organisms:	National Collections of Industrial and Marine Bacteria Ltd., Aberdeen, UK

4. Environmental conditions:

Temperature:	27.0±0.5°C

5. Experimental dates: May 11, 2000 (first run) and May 17, 2000 to May 18, 2000

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate on *Pseudomonas putida* growth inhibition were evaluated in a 16-hour static toxicity test. The test concentrations of 1.0, 3.2, 10, 32, and 100 mg/L in test medium were prepared in duplicate and sterile conditions in conical flasks. These test solutions were prepared by adding the appropriate amount of a 500 mg/L stock solution (0.125 g glyphosate in 250 mL deionised water) directly into the flasks. Flasks containing 1.0, 3.2, 10, 32, and 100 mg/L (single replicates) of the reference toxic substance (3,5-dichlorophenol) and three control flasks were also prepared. Four mL growth medium, 1 mL inoculum and deionised water were added to obtain a final volume of 50 mL test solution. After shaking at 27.0±0.5°C (in an in incubator) at 150 rpm for 16±1 hours the optical density of the contents of each flask were measured at 600 nm with a Uvikon 930 spectrophotometer.

II. RESULTS AND DISCUSSION

A. FINDINGS

The effects of glyphosate on *Pseudomonas putida* are shown below.

Table B.9.8-1: Effects of glyphosate on *Pseudomonas putida*

Nominal concentration [mg test item/L]	Mean optical density	Mean % inhibition
Control	0.859	-0
1.0	0.836	3
3.2	0.838	2
10	0.842	2
32	0.868	0
100	0.878	0
3,5-DCP 1.0	0.839	2
3,5-DCP 3.2	0.857	0
3,5-DCP 10	0.851	1
3,5-DCP 32	0.055	94
3,5-DCP 100	0.047	95

B. OBSERVATIONS

Based on the obtained results, the IC_{50} is > 100 mg/L and the highest concentration at which no effect was observed (NOEC) to be 100 mg/L. The reference substance 3,5-dichlorophenol gave an IC_{50} of 18 mg/L.

The following validity criterion was fulfilled according to the guideline:

- The EC_{50} of the reference substance 3,5-dichlorophenol was between 10 mg/L and 30 mg/L (actual value: 18 mg/L)

The following points deviated from the current guideline requirements:

- The inoculum concentration was given as 0.532. Then 1 mL of this inoculum was added to each final 50 mL test solution (including the control solution). The control inoculum concentration was measured as 0.859 at the end of the test but the initial optical density of the control solution was not provided, so it is not possible to conclude on the study validity according to guideline requirements.
- Only two replicates were setup for each test item dilution. The guideline requires three parallel batches for each dilution step.
- The test solutions were maintained at 27±0.5°C for 16 hours instead of 23±1°C.

It is not possible to conclude on the study validity, regarding the requested control inoculum multiplication factor so the study is not considered as valid for the risk assessment.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The 16-h IC_{50} for *Pseudomonas putida* exposed to glyphosate technical was >100 mg a.e./L based on nominal concentration. The NOEC after 16 h was 100 mg a.e./L.

It is not possible to conclude on the study validity regarding the requested control inoculum multiplication factor so the study is considered invalid for risk assessment purposes. Nevertheless, the results of the study are in line with the additional sludge study (CA 8.8/002)

Assessment and conclusion by RMS:

Test item: glyphosate technical

As previously highlighted in RAR 2015, the validity criteria according to ISO 10712 were not fulfilled, as the control inoculum may have not multiplied by a factor of at least 60 within the test period. RMS then agrees with the applicant that the study is invalid for risk assessment purposes.

The study is considered invalid.

Data point:	CA 8.8/002
Report author	[redacted]
Report year	1990
Report title	Assessment of the acute toxicity of glyphosate technical on aerobic waste-water bacteria
Report No	277830
Document No	-
Guidelines followed in study	OECD No.209 (1984)
Deviations from current test guideline identified by the applicant: **See RMS analysis in RMS comment box**	*Deviation from the guideline OECD 209 (2010):* *Minor:* *- Only one replicate in each treatment concentration* *- No indication on the dissolved oxygen concentration*
Previous evaluation	Yes, accepted in RAR (2015)
GLP/Officially recognised testing facilities	Yes
Acceptability/Reliability (RMS)	Valid

Executive Summary

The effects of glyphosate technical on activated sludge were determined in a 3-hour exposure laboratory study. Activated sludge from a domestic waste-water treatment plant was exposed to the test item at concentrations of 3.2, 10, 32, 50, and 100 mg./L, 2 untreated controls and a toxic reference (3,5-dichlorophenol at concentrations of 1.0, 3.2, 10, 32, and 50 mg/L). After 180 minutes of aeration at 22°C, samples were taken from the test flasks for oxygen measurement over a period of up to 10 minutes. The inhibitory effect of the test item is expressed as oxygen consumption per minute.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Glyphosate technical
Aspect:	White solid
Lot/Batch #:	229-Jak-5-1
Purity:	98.9%

2. Vehicle of test material and positive control: Vehicle: distilled water
Positive control 3,5-dichlorophenol

3. Test system:

Test system:	Activated sludge
Source:	Domestic waste-water treatment plant (ARA, Sissach, Switzerland
Nutrient solution:	Synthetic sewage feed
Dry sludge concentration:	4 g/L
Test vessel:	500 mL glass flasks

4. Environmental conditions:

Temperature:	20-25°C until use. 22°C during the test.
pH:	7.5-7.7

5. Experimental dates: July 19, 1990 (3 hours duration)

B: STUDY DESIGN AND METHODS

1. Experimental treatments: The effects of glyphosate technical on activated sludge were determined in a 3-hour exposure laboratory study. Activated sludge from a domestic waste-water treatment plant was exposed to the test item at concentrations of 3.2, 10, 32, 50, and 100 mg/L, 2 replicates of untreated controls and a toxic reference (3,5-dichlorophenol at concentrations of 1.0, 3.2, 10, 32, 50, and 100 mg/L). A stock solution of 500 mg/L was prepared by dissolving glyphosate in distilled water. The sludge was sieved, centrifuged and the solid material resuspended in tap water and again centrifuged. This procedure was repeated a further 2 times. An aliquot of the final sludge suspension was made up with Soerensen buffer to 1 liter. To that mixture, 50 mL OECD recommended synthetic sewage feed were added.

Glass flasks were filled with appropriate aliquots of stock solutions, water and activated sludge up to 500 mL final volume and aerated with an air flow of about 0.2 L/minute.

2. Observations: After 180 minutes of aeration at 22°C, samples were taken from the test flasks for oxygen measurement over a period of up to 10 minutes. The inhibitory effect of the test item is expressed as oxygen consumption per minute. Respiration rate was expressed as percent inhibition relative to the control.

3. Statistical calculations: EC values were calculated using linear regression.

II. RESULTS AND DISCUSSION

A. FINDINGS

The influence of glyphosate on oxygen consumption of activate sludge is presented below.

Table B.9.8-2: Influence of glyphosate on oxygen consumption of activate sludge

Nominal concentration [mg test item/L]	Oxygen consumption [mg O_2 per litre per min]	Mean [deviation]	Inhibition [%]
Control	1.02	1.085 (12.7%)	-
Control	1.15		-
3.2	1.16	-	-6.9
10	1.09	-	-0.5
32	1.15	-	-6.0
50	1.09	-	-0.5
100	1.17	-	-7.8
3,5-DCP 1.0	1.11	-	-2.3
3,5-DCP 3.2	1.07	-	1.4
3,5-DCP 10	0.38	-	65.0
3,5-DCP 32	0.07	-	93.5
3,5-DCP 50	0.05	-	95.4

B. OBSERVATIONS

No inhibition of the respiration rate of the sludge was observed (-7.8%) at the highest concentration of glyphosate of 100 mg a.e./L. The EC_{50} for the toxic reference 3,5-DCP was found to be 8.6 mg/L.

The validity criteria were fulfilled according to OECD 209 (2010):

- the coefficient of variance for oxygen uptake in the control replicates was not more than 30% (actual value: 12.7%)
- the EC_{50} of 3,5-dichlorophenol was in the expected range (actual value: 8.6mg/L)
- Control oxygen uptake rate was more than 20 mg/g of activated sludge (dry weight of suspended solids) in an hour. Based on 4 g/L dry sludge concentration with a dilution ratio of 200mL in 500mL final solution and oxygen uptake of 1.085 mgO_2/L.min.

The following points deviated from the current guideline requirements but are not expected to have impact on the study validity:

- Only one replicate in each treatment concentration instead of 5 replicates.
- No indication on the dissolved oxygen concentration. It should be maintained above 60 – 70% saturation. The air-flow was 0.2 L/min instead of 0.5 to 1 L/min recommended due to foam.

III. CONCLUSIONS

Assessment and conclusion by applicant:

The EC_{50} for waste-water micro-organisms exposed to glyphosate was determined to be >100 mg/L. The NOEC for waste-water micro-organisms exposed to glyphosate was determined to be 100 mg/L.

This study is considered valid and the EC_{50} > 100 mg a.e./L and the NOEC of 100 mg a.e./L can be used in the risk assessment for micro-organisms exposed to glyphosate technical.

Assessment and conclusion by RMS:

Test item: glyphosate technical

The test is regarded to be valid since the control oxygen uptake rate criteria was fulfilled (RMS calculation: 26 i.e. >20 mg oxygen/g of activated sludge d.w. in an hour) and the variance between the control samples was <30% (12.7 %). The toxic reference performed well (validity criteria fulfilled).

RMS could not find any information on any potential pH adjustement of the tested solutions (RMS guesses this may have occurred in view of the high concentrations that were tested and the homogeneous pH values measured for all different concentrations). Nevertheless, RMS cannot exclude a buffering action of the sludge neither.
As noted by the applicant, only one replicate was used in each treatment concentration. RMS nevertheless considers the results reliable enough as no effect were observed for all tested concentrations. In the absence of effect, RMS considers that a NOEC can reasonably be set.
The applicant also notes that the air-flow was 0.2 L/min instead of 0.5 to 1 L/min recommended due to foam. RMS agrees that the deviation is minor.

EC50 for waste-water micro-organisms exposed to glyphosate >100 mg/L.

B.9.9. MONITORING DATA

No monitoring data concerning adverse effects of the active substance to non-target organisms has been submitted.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

AMPA does not have a comparable target activity as the parent active compound as it does not contain the functional moiety to cause the herbicidal action that glyphosate does (SANCO221/2000 – rev 10) and is not expected to impact the Shikimic acid pathway.

Studies available on alga and aquatic macrophytes suggested that AMPA is 14 times less toxic than glyphosate on alga and 7 times less toxic on aquatic macrophytes.

In addition to differences in the mode of activity described above, the relative herbicidal activity between AMPA and glyphosate was reported ([redacted] 2012 CP 10.6.2/004). In this report, Relative post-emergence phytotoxicity between glyphosate and aminomethylphosphonic acid (AMPA) were compared for the following species:9 Dicotyledons: (cocklebur, hemp sesbania, lambsquarters, morning glory, smartweed, soybean, sugar beet, velvetleaf, wild buckwheat) and 8 Monocotyledons: (barnyard grass, corn, crabgrass, green foxtail, proso millet, rice, sorghum, wheat).
EC50 molar ratios were calculated as EC50 AMPA/ EC50 glyphosate acid and ranged from 3.4 for hemp sesbania to 86.8 for common lambsquarters. In all cases, the ratios were greater than two, indicating that AMPA has less than 50% of the herbicidal activity of glyphosate. The endpoints presented above cannot be used for the risk assessment of non-target plants but are indicative of a lower toxicity of AMPA.
RMS considered that AMPA has lost the herbicidal activity of the parent glyphosate.
The herbicidal activity of AMPA is considered to be below 50 % of the parent activity .

B.9.11. REFERENCES RELIED ON

B.9.11.1. Literature search

B.9.11.1.1. Summary

Article 8(5) of Regulation (EC) No 1107/2009 requires applicants submitting dossiers for approval of active substances to provide relevant scientific peer reviewed open literature. This summary of scientific peer reviewed open literature conforms to EFSA guidance "Submission of scientific peer-reviewed open literature under Regulation (EC) No 1107/2009, EFSA Journal 2011; 9(2):2092".

Peer reviewed open literature containing data and analysis dealing with the side effects on health, environment, and non-target species for common name, and its relevant metabolites. The data published within the last ten years before the date of the submission of glyphosate (renewal) dossier were reviewed. This initial search was reported in the Literature Review Report of May 2020.
Upon request of RMS, an additional literature search covering the period from January to June 2020 has been conducted. This additional search was reported in the Literature Review Report of October 2020.

For detailed summary of literature search, please refer to Volume 1 level 2 under the chapter "Summary of methodology proposed by the applicant for literature review and for all sections".

B.9.11.1.2. Search strategy

B.9.11.1.2.1. Date of the search – online search service used when applicable

The applicant has performed the search via the online service provider STN (www.stn-international.de).

The following databases have been used in order to cover the requirements of the EFSA Guidance Document: AGRICOLA, BIOSIS, CABA, CAPLUS, EMBASE, ESBIOBASE, MEDLINE, TOXCENTER, FSTA, PQSCITECH, and SCISEARCH.

Due to a large amount of public literature available for the active substance glyphosate, the search has been divided into six parts. As the number of records returned by a "single concept search" was extremely large for the searches Part 0, Part 1, Part 2, Part 3 and Part 5b, a "focused search for grouped data requirements" have been performed (a combination of a substance search and "search filters" defined for the four relevant sections – ecotoxicology, toxicology, environmental fate, and residues).
A "single concept search" was used for the searches Part 4, Part 5a and Part 6.

In October 2020, upon request of RMS, an additional literature search covering the period from January to June 2020 has been conducted.

The table below summarises the seven search parts that cover the period from January 2010 to June 2020.

Overview of the searches conducted for glyphosate and its metabolites

Search	Performed for	Covering publication period	Conducted on
Part 0	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2010 – Dec 2011	28th Oct 2019
Part 1	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2012 – Dec 2017	08th Jun 2018.
Part 2a	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jan 2018 – Dec 2018	04th Jul 2019
Part 2b		Jan 2019 – Jun 2019	10th Jul 2019
Part 3	glyphosate, AMPA, N-acetyl-AMPA and N-acetyl-glyphosate	Jul 2019 – Dec 2019	7th Jan 2020
Part 4	HMPA	Jan 2010 – Feb 2020	24th Feb 2020
Part 5a	N-methyl-AMPA, N-glyceryl-AMPA, N-malonyl-AMPA	Jan 2010 – Feb 2020	27th Feb 2020
Part 5b	methylphosphonic acid	Jan 2010 – Feb 2020	27th Feb 2020
Part 6	N-methylglyphosate	Jan 2010 – April 2020	04th May 2020
Additional search upon RMS request	Glyphosate AMPA N-acetyl-AMPA N-acetyl-glyphosate HMPA N-methyl-AMPA N-glyceryl-AMPA N-malonyl-AMPA methylphosphonic acid N-methylglyphosate	January 2020 – June 2020 (incl. June 2020)	02-July 2020

AMPA = (aminomethyl)phosphonic acid
HMPA = (hydroxymethyl)phosphonic acid

B.9.11.1.2.2. Time window of the literature search

Please refer to B.9.11.1.2.2.

B.9.11.1.2.3. Bibliographic Databases used in the literature review

Databases	Frequency of updates
AGRICOLA	Monthly
BIOSIS	Weekly
CABA	Weekly
CAPLUS	Daily updates bibliographic data; weekly updates indexing data
EMBASE	Daily
ESBIOBASE	Weekly

MEDLINE	Six times each week, with an annual reload
TOXCENTER	Weekly
FSTA	Weekly
PQSCITECH	Monthly
SCISEARCH	Weekly

B.9.11.1.2.4. Input parameters for literature search

Substance name	Glyphosate Salts: isopropylamine, potassium, ammonium, methylmethanamine
IUPAC name	2-(phosphonomethylamino)acetic acid
Other names given to the substance/trade name	
EC number	
CAS number	1071-83-6 Salts: 38641-94-0, 70901-12-1, 39600-42-5, 69200-57-3, 34494-04-7, 114370-14-8, 40465-66-5, 69254-40-6

metabolite	AMPA
IUPAC name	(aminomethyl)phosphonic acid
Other names given to the substance/trade name	
EC number	
CAS number	1066-51-9

metabolite	N-acetyl glyphosate
IUPAC name	N-acetyl-N-(phosphonomethyl)glycine
Other names given to the substance/trade name	
EC number	
CAS number	129660-96-4

metabolite	N-acetyl AMPA
IUPAC name	[(acetylamino)methyl]phosphonic acid
Other names given to the substance/trade name	
EC number	
CAS number	57637-97-5

metabolite	HMPA
IUPAC name	(hydroxymethyl)phosphonic acid
Other names given to the substance/trade name	
EC number	
CAS number	2617-47-2

metabolite	N-methyl AMPA
IUPAC name	[(methylamino)methyl]phosphonic acid
Other names given to the substance/trade name	
EC number	
CAS number	35404-71-8

metabolite	N-glyceryl AMPA
IUPAC name	(2,3-dihydroxypropanoylamino)methylphosphonic acid
Other names given to the substance/trade name	
EC number	
CAS number	No data

metabolite	N-malonyl AMPA
IUPAC name	3-oxo-3-(phosphonomethylamino)propanoic acid
Other names given to the substance/trade name	
EC number	
CAS number	no data

metabolite	methylphosphonic acid
IUPAC name	methylphosphonic acid
Other names given to the substance/trade name	
EC number	
CAS number	993-13-5

metabolite	N-methylglyphosate
IUPAC name	2-[methyl(phosphonomethyl)amino]acetic acid
Other names given to the substance/trade name	
EC number	
CAS number	24569-83-3

B.9.11.1.2.5. Endpoint-specific search terms

The approach used for the searches was either the "single concept search" (in searches Part 4, 5a and 6 of Literature Review report of May 2020) or the "focused search for grouped data requirements" (in searches Part 0, 1, 2, 3, 5b of Literature Review report of May 2020, and searches from literature review Report of October 2020), which combines the active substance / metabolites keywords with the search filters used in the technical sections.

Following the approach "focused search for grouped data requirements", the search filters defined for ecotoxicology section did not contain keywords related to endpoints such as NOEL, NOAEL, LC50, EC50, EC10 (…). However, this is not seen as critical by RMS as the terminology used allow to retrieve studies that proposed endpoints.

Ecotoxicology [Gly1] OR [Gly2] OR [Gly3] OR [Gly4] OR [Gly5] AND the following search filters
tox? OR ecotox? OR ?toxic OR ?toxicity OR hazard OR adverse OR endocrine disrupt? OR bioaccumulate? OR biomagnifi? OR bioconcentration OR poison OR effect OR indirect effect? OR direct effect? OR biodivers? OR protection goals OR eco? OR impact OR population OR OR community OR wildlife OR incident OR wildlife OR incident OR pest OR bird? OR acute OR chronic OR long-term OR mallard OR duck OR quail OR bobwhite OR Anas? OR Colinus? OR wild OR dietary OR aquatic OR fish OR daphni? OR alg? OR chiron? OR sediment dwell? OR benthic OR lemna OR marin? OR estuarine OR crusta? OR gastropod? OR insect OR mollusc OR reptile OR amphib? OR plant AND submerge? OR emerge? OR bee? OR apis OR apidae OR bumble? OR colony OR hive OR pollinator OR solitary OR alg? OR aquatic OR freshwater OR vertebrat? OR mammal? OR rat OR mouse OR mice OR rabbit OR hare OR protection OR model? OR vole OR pest OR arthropod? OR beneficials OR typhlodromus OR aphidius OR parasitoid OR predator OR chrysoperla OR Orius OR spider OR worm? OR ?worm OR Eisenia OR soil OR collembol? OR macro organism OR folsomia OR springtail OR decompos? OR micro organisms OR microorganisms OR microbial OR carbon OR nitrogen OR plant? OR vegetative vigo? OR seedling OR germination OR monocot? OR dicot? OR sewage OR activated sludge OR biodegrad? OR bioaccumulation? OR amphib? OR reptile? OR aquatic plant OR beneficial

B.9.11.1.2.6. Filters

Keywords used for the active substance glyphosate and its metabolites

Gly1: Glyphosate and AMPA	glyphosat? OR glifosat? OR glyfosat? OR 1071-83-6 OR 38641-94-0 OR 70901-12-1 OR 39600-42-5 OR 69200-57-3 OR 34494-04-7 OR 114370-14-8 OR 40465-66-5 OR 69254-40-6 OR aminomethyl phosphonic OR aminomethylphosphonic OR 1066-51-9
Gly2: N-acetyl glyphosate and N-acetyl AMPA	2 acetyl phosphonomethyl amino acetic acid OR n acetyl glyphosate OR n acetylglyphosate OR n acetyl n phosphonomethyl glycine OR 129660-96-4 OR n acetyl ampa OR acetylamino methyl phosphonic acid OR acetylaminomethyl phosphonic acid OR 57637-97-5
Gly 3: HMPA	2617-47-2 OR hydroxymethanephosphonic acid OR hydroxymethyl phosphonate OR hydroxymethylphosphonate OR hydroxymethyl phosphonic acid OR hydroxymethylphosphonic acid OR methanehydroxyphosphonic acid OR phosphonic acid(1w)hydroxymethyl OR phosphonomethanol
Gly 4: N-methyl AMPA	35404-71-8 OR methylamino methyl phosphonic acid OR methylaminomethyl phosphonic acid OR methylaminomethylphosphonic acid OR n methyl ampa OR nsc 244826 OR phosphonic acid methylamino methyl OR phosphonic acid p methylamino methyl

Gly 4: N-glyceryl AMPA	2 3 dihydroxy 1 oxopropyl aminomethyl phosphonic acid OR 2 3 dihydroxy 1 oxopropyl aminomethylphosphonic acid OR n glyceryl ampa
Gly 4: N-malonyl AMPA	3 oxo 3 phosphonomethyl amino propanoic acid OR 3 oxo 3 phosphonomethyl aminopropanoic acid OR n malonyl ampa
Gly 4: methylphosphonic acid	993-13-5 OR dihydrogen methylphosphonate OR methanephosphonic acid OR methyl phosphonic acid OR methylphosphonic acid OR nsc 119358 OR phosphonic acid methyl OR phosphonic acid p methyl
Gly 5: N-methylglyphosate (NMG)	24569-83-3 OR 2 methyl phosphonomethyl amino acetic acid OR 2 methyl phosphonomethyl aminoacetic acid OR acetic acid 2 n methyl n phosphonatomethyl amino OR glycine n methyl n phosphonomethyl OR glyphosate n methyl OR methyl glyphosate OR methyl phosphonomethyl amino acetic acid OR methyl phosphonomethyl aminoacetic acid OR n methyl n phosphonomethyl glycine OR n methylglyphosate OR n phosphonomethyl n methyl glycine OR n phosphonomethyl n methylglycine

(1w) = proximity operator (this order, up to 1 word between)
AND / OR / NOT = boolean search operators
? = any character(s)

B.9.11.1.3. Search results

The table below summarised the number of published papers resulted from the search based on the criteria described above as proposed by the applicant. RMS did a complete re-analysis of title, abstract and/or full text and proposed.

Summary of the literature review (applicant)

Section	**Number of articles found**	**Rapid assessment (title/abstract level)**		**Detailed assessment (full-text level)**	
		non-relevant articles	**potentially relevant / unclear relevance**	**non-relevant articles**	**relevant articles (category A+B+C)**
Ecotoxicology (initial search)	1464	918	546	398	148
Ecotoxicology (additional search)*	150	121	29	14	15
Total Ecotoxicology	**1614**	**1039**	**575**	**412**	**163**

* number of published papers identified upon removal of duplicates within the additional search (January 2020 – June 2020) and articles found already in the initial search.

The relevant articles after full-text assessment is provided in the table below.

Relevant articles by full text level – according to the EFSA GD, Point 5.4.1 (applicant)

	Relevant articles by full-text (EFSA GD, Point 5.4.1)*		
Section	**Category A***	**Category B***	**Category C***
Ecotoxicology (initial search)	10	135	3
Ecotoxicology (additional search)	2	13	0

Total (Ecotoxicology)	**12**	**148**	**3**

*Category A = relevant articles, Category B = relevant but supplementary articles, Category C = articles of unclear relevance.

B.9.11.1.4. Evaluation

After combination of searches and removal of duplicates, the remaining articles were assessed for their relevance at title / abstract level (so-called rapid assessment). Articles that were identified as "non-relevant" in the rapid assessment were excluded from further evaluation. For articles that were not excluded in the rapid assessment, full-text documents were reviewed.

The following criteria has been used by the applicant for the selection of relevant studies (strategy applied for all sections):

- Relevance assessment at "title / abstract" level (applicant)

Criteria applied for "non-relevance" at "title / abstract" level by the applicant for all sections:

- Publications related to efficacy (resistance related articles, new uses of control of pest/crops) or to agricultural / biological research (crop science, breeding, fertilization, tillage, fundamental plant physiology / micro / molecular biology).
- Publications dealing with analytical methods / development.
- Publications describing new methods of synthesis (discovery / developments) or other aspects of basic (organic / inorganic) chemistry.
- Patents.
- Wastewater treatment.
- Abstracts referring to a conference contribution that does not contain sufficient data / information for risk assessment.
- Publications focusing on genetically modified organisms / transgenic crops; no data directly relevant to glyphosate evaluation (e.g. crop compositional analysis, gene flow, protein characterization).
- Publications where glyphosate or a relevant metabolite were not the focus of the paper.
- Secondary information including scientific and regulatory reviews8.
- Articles dealing with political / socio / economic analysis.
- Observations caused by mixture of compounds / potentially causal factors and thus not attributable to a substance of concern (e.g. mixture toxicity).
- Study design, test system, species tested, exposure routes etc. are not relevant for the European regulatory purposes.
- Findings related to ecotoxicology, toxicology, metabolism, environmental fate.
- Publications not dealing with EU representative uses / conditions (e.g. field locations, soil properties, non-EU monitoring etc.).

- Relevance assessment at "full-text" level (applicant)

Criteria applied for "non-relevance" at "full-text" level

- Publications dealing with a Roundup formulation that is not the representative formulation for the AIR5 dossier in Europe.
- Publications dealing with general pesticide exposures (not glyphosate specific).
- The presented endpoints are not relatable to the EU level risk assessment.
- Opinion articles where no new data is provided that can be used for risk assessment.
- Findings based on cellular and molecular level that cannot be related to the risk assessment.
- Criteria outlined in above for for "non-relevance" at "title / abstract" level", that needed the full text document to determine.

Other considerations by the applicant:
Many articles that have been considered relevant for the risk assessment of glyphosate and have been assessed for reliability on full text basis contain experimental data as well on glyphosate as such as on formulations (different from MON 52276) and co-formulants. In such case only the toxicology data pertinent to glyphosate and to the reference formulation (if that can be clearly stated by the author of the

article) are summarized and discussed. In the case of articles on exposure monitoring and epidemiology, exposure to glyphosate formulations are considered.

- Categorization of "relevant" articles at full text level (applicant)

Articles that have been identified as "relevant" in the rapid assessment have been categorized as recommended in the EFSA GD 2011; 9(2):2092, Point 5.4.1.

- Category (a) Studies that provide data for establishing or refining risk assessment parameters. These studies should be summarised in detail following the subsequent steps of the OECD Guidance documents (OECD, 2005; 2006) and should be considered for reliability.
- Category (b) Studies that are relevant to the data requirement, but in the opinion of the applicant provide only supplementary information that does not alter existing risk assessment parameters. After expert judgement, essential reliability parameters affect the full reliability of the study. A justification for such a decision should be provided.
- Category (c) Studies for which relevance cannot be clearly determined. For each of these studies the applicants should provide an explanation of why the relevance of such studies could not be definitively determined.

The following criteria has been used by the applicant for reliability assessment
For articles, which have been identified as category A, under the Point 5.4.1 of the EFSA GD document, a reliability assessment has been performed. The reliability criteria for ecotoxicology section are summarized in the table below.
For articles (category A) that have been identified as reliable or reliable with restrictions, summaries have been compiled. Articles of category A which have been identified as non-reliable were downgraded to articles of category B (relevant but supplementary).

Applied for	Reliability criteria
Ecotoxicology, Environmental Fate, Residues	For guideline-compliant studies (GLP studies): OECD, OPPTS, ISO, and others. The validity/quality criteria listed in the corresponding guidelines are met.
Ecotoxicology, Environmental Fate, Residues	(No) previous exposure to other chemicals is documented (where relevant).
Ecotoxicology	For aquatic studies, the test substance is dissolved in water or where a carrier is required, it is appropriate (non-toxic) and a carrier control / positive control is considered in the test design.
Environmental Fate, Residues	The test substance is dissolved in water or non-toxic solvent.
Ecotoxicology, Environmental Fate, Residues	Test item is sufficiently documented, and reported (i.e. purity, source, content, storage conditions).
Ecotoxicology	For tests including vertebrates, compliance of the batches used in toxicity studies compared to the technical specification.
Ecotoxicology	Species used in the experiment are clearly reported, including source, experimental conditions (where relevant): strain, adequate age/life stage, body weight, acclimatization, temperature, pH, oxygen (dissolved oxygen for aquatic tests) content, housing, light conditions, humidity (terrestrial species) incubation conditions, feeding.
Ecotoxicology	The validity criteria from relevant test guidelines can be extrapolated across different species but not necessarily across different test designs. If different, then the nature of the difference and impact should ideally be discussed.
Ecotoxicology, Environmental Fate, Residues	Only glyphosate or its metabolites is the test substance (excluding mixture), and information on application of the test substance is described.
Ecotoxicology, Environmental Fate, Residues	The endpoint measured can be considered a consequence of glyphosate (or a glyphosate metabolite).
Ecotoxicology, Environmental Fate, Residues	Study design / test system is well described, including when relevant: concentration in exposure media (dose rates, volume applied, etc.), dilution/mixture of test item (solvent, vehicle) where relevant.
Ecotoxicology, Environmental Fate, Residues	Analytical verifications performed in test media (concentration) / collected samples, stability of the test substance in test medium should be documented.
Ecotoxicology	The test has been performed in several dose levels (at least 3) including a positive / negative control where relevant.
Ecotoxicology	Suitable exposure throughout the whole exposure period was demonstrated and reported.
Ecotoxicology	A clear concentration response relationship is reported – in studies where the dose response test design is employed.
Ecotoxicology	A sufficient number of animals per group to facilitate statistical analysis reported: mortality in control groups reported, observations/findings in positive/negative control clearly reported (where relevant).
Ecotoxicology, Environmental Fate, Residues	Assessment of the statistical power of the assay is possible with reported data.
Ecotoxicology, Environmental Fate, Residues	Statistical methodology is reported (e.g., checking the plots and confidence intervals).

Applied for	Reliability criteria
Ecotoxicology	Description of the observations (including time-points), examinations, and analyses performed, with (where relevant) dissections being well documented.
Ecotoxicology	For terrestrial ecotoxicological studies in the laboratory or the field, the substrates used should be adequately described e.g. nature of substrate i.e. species of leaf or soil type.
Ecotoxicology, Environmental Fate, Residues	Field locations relevant / comparable to European conditions.
Ecotoxicology, Environmental Fate, Residues	Characterization of soil: texture (sandy loam, silty loam, loam, loamy sand), pH (5.5-8.0), cation exchange capacity, organic carbon (0.5-2-5%), bulk density, water retention, microbial biomass (~1% of organic carbon).
Ecotoxicology, Environmental Fate	Other soils where information on characterization by the parameters: pH, texture, CEC, organic carbon, bulk density, water holding capacity, microbial biomass.
Ecotoxicology, Environmental Fate, Residues	For tests including agricultural soils, they should not have been treated with test substance or similar substances for a minimum of 1 year.
Ecotoxicology, Environmental Fate	For soil samples, sampling from A-horizon, top 20 cm layers; soils freshly from field preferred (storage max 3 months at 4 +/- 2°C).
Ecotoxicology, Environmental Fate, Residues	Data on precipitation is recorded.
Environmental Fate	The temperature was in the range between 20-25°C and the moisture was reported.
Environmental Fate	The presence of glyphosate identified in samples were collected from European groundwater, soil, surface waters, sediments or air.
Ecotoxicology	For lab terrestrial studies, the temperature was appropriate to the species being tested and generally should fall within the range between 20-25°C and soil moisture / relative humidity was reported.
Ecotoxicology	For bee studies, temperature of the study should be appropriate to species.
Ecotoxicology	For lab aquatic studies:
	The source and / or composition of the media used should be described.
	The temperature of the water should be appropriate to the species being tested and generally fall within the 15-25°C.
Ecotoxicology, Residues	The residue data can be linked to a clearly described GAP table, appropriate in the context of the renewal of approval of glyphosate (crop, application method, doses, intervals, PHI).
Ecotoxicology, Environmental Fate, Residues	Analytical results present residues measurements which can be correlated with the existing residues definition of glyphosate, and where relevant its metabolites.
Ecotoxicology, Environmental Fate, Residues	Analytical methods are clearly described; and adequate statement of specificity and sensitivity of the analytical methods is included.
Ecotoxicology	Assessment of the ECX for the width of the confidence interval around the median value; and the certainty on the level of protection offered by the median ECX is reported.
Environmental Fate	Radiolabel characterization: purity, specific activity, location of label is reported.
Environmental Fate	If degradation kinetics are included: data tables / model description / statistical parameters for kinetic fit to be provided.
Environmental Fate, Residues	Monitoring data: description of matrix analysed, and analytical methods to be fully described.
Environmental Fate	Clear description of application rate and relevance to approved uses.
Overall assessment: Reliable / Reliable with restrictions / Not reliable	

RMS analysis

From the initial check of the literature search submitted in June 2020, RMS has checked the literature search, the lists of studies and Excel sheet for studies that were not submitted by the GRG. For the 918 articles that were deemed "non-relevant after rapid assessment", this check was based on the title only. For the 546 articles considered "potentially relevant/unclear relevance", the justification provided by the applicant was checked. When justification was not convincing, the abstract was also checked. When justification pointed major drawbacks these were not considered further by RMS and no summary is required. When adverse effect are observed at low doses/concentrations, RMS also requires summaries

for potential use in an WoE approach (endocrine disruption, species sensitivity, sensitive growth stage, sublethal effects,…).
Observations related to transgenic crops were not considered as the environmental impact of the crop itself is already controversial, except if effects can be related to glyphosate itself.
For the additionnal literature search requested by RMS, the same principles were followed.

Following this initial check of the literature search submitted in June 2020, the applicant was requested to:

- provide study summaries for 51 articles of category B (from table 34 of initial Literature Review Report, May 2020),
- provide study summaries for 3 articles from category C (from table 36 of initial Literature Review Report, May 2020),
- provide study summaries for 46 studies excluded after detailed assessment by the applicant (from table 38 of initial Literature Review Report, May 2020)
- and to provide full-text and study summaries for 91 articles considered as non-relevant after a rapid assessment (from Excel file provided with the initial Literature Review Report, May 2020, filtered in 'final section' on ecotoxicology).

When assessing the literature review, RMS has considered in particular that:

- Studies that were conducted with other formulations may provide useful information that can be considered in a weight of evidence approach.
- Endpoints not relatable to the EU risk assessment might provide relevant information in a weight of evidence approach. RMS then does not consider it should be used solely as a criteria for "non-relevance".
- Regarding the reliability criteria: The criteria used by the applicant to state on the reliability of each study (see tables above) are considered too restrictive for literature data. Indeed the reliability assessment is close to the one used for studies conducted according to test guidelines such OECD/ISO standards (for example, withdraw of studies with no analytics, not GLP…). However, this may exclude many studies potentially usable in a weight of evidence approach.

The approach used by RMS to assess the relevance and reliability of literature studies can be found in the introduction part of the appendix to Volume 3 CA B.9 related on literature data on ecotoxicology.

Table B.9.11.1.4-1 : Results of the article selection process for ecotoxicology (RMS)

Summary of the review	Number	Justification
Total number of summary records retrieved from search		
Total number of summary records retrieved after removing duplicates from all database searches	1614	
Number of summary records excluded after rapid assessment for relevance (by title/abstract)	1130	1039 identified by applicant + 91 articles requeted by RMS (see above)
Number of studies excluded from the risk assessment after detailed assessment of full-text documents (i.e. not relevant)	*	See Table B.9.11.1-2.
Number of studies not excluded for relevance after detailed assessment (i.e. reliable studies and studies of unclear reliability)	*	See Table B.9.11.1-3. and B.9.11.1-4
Number of studies included in the RAR/DAR as supporting information	*	

* As several data gaps have been identified in Table B.9.11.1-2, the definitive numbers of each category will be precised at a later stage.

Only one study on terrestrial vertebrates (*i.e.*, Daam et al. 2019) was considered as relevant by the applicant after the literature search in June 2020 (report no. 108689-CA9-1), while 6 studies were considered 'relevant but supplementary'. Regarding non target organisms other than terrestrial vertebrates, nine studies were considered as relevant by the applicant after the literature search in June

2020, while 129 studies were considered 'relevant but supplementary'. However, the RMS has identified several other potentially relevant studies in the literature search report and has thus requested that further 158 studies among which 33 studies on birds, amphibians and reptiles should be summarised and provided by the applicant. Moreover, after the top-up literature search (report no. 113898-CA9-1), one study on terrestrial vertebrates was identified by the applicant as being relevant (*i.e.*, Turhan et al. 2020), while 6 studies were considered not relevant after detailed assessment (see Table 9.11.1-2). The RMS considers some of these 6 studies to be potentially relevant for the assessment and has thus listed them as data gaps. Regarding non target organisms other than terrestrial vertebrates, one study was considered as relevant by the applicant after the top-up literature search, while 7 studies were considered 'relevant but supplementary'. From the total of 35 studies on terrestrial vertebrates summarised in the RAR (see Appendix to Vol 3CA), the RMS has excluded 9 studies after detailed assessment, and kept the remaining 26 studies as 'relevant' (9 studies) and 'less relevant but supplementary' (17 studies). A total of 97 studies on non target organisms other than vertebrates have been summarised in the RAR (see Appendix to Vol 3CA). From those 97 studies, 2 studies (cited 5 times) presented results of regulatory studies. Among the 92 remaining studies, the RMS has excluded 58 studies after detailed assessment, and kept the remaining ones as 'relevant' (24 studies) and 'less relevant but supplementary' (10 studies). Several studies for which detailed assessment was required have been listed as data gap (see Table 9.11.1-2).

RMS has **identified several** articles considered as non-relevant after a rapid assessment by the applicant (from Excel file provided with the additional Literature Review Report, October 2020, filtered in 'final section' on ecotoxicology) and other identified based on RMS knowledge for which further consideration and more detailed study summaries should be requested in order to complete the evaluation. A data gap is set for the applicant to provide the study reports of the listed studies together with a study summary and assessment of relevance and reliability.

Table 9.11.1.4-2: List of literature data of rapid assessment (or identified based on RMS knowledge) to be provided and summarised by the applicant

Reference	Year	Title	Publication journal	Study overview and RMS justification
Quassinti L. et al.	2015	Toxicity of Cupside 480SL® Spray Mixture Formulation Of Glyphosate To Aquatic Organism	Pesticide Biochemistry and Physiology, Vol. 93, pp. 91-95	Study from the literature review on Endocrine Dirusption not found in the general literature review on ecotoxicology data. Needed to assess its relevance/reliabity. Data gap: Provide the study report together with a study summary including detailed assessment of relevance and reliability.
Fanton Noelia Bacchetta Carla Rossi Andrea Gutierrez Maria Florencia	2020	Effects of a glyphosate-based herbicide on the development and biochemical biomarkers of the freshwater copepod Notodiaptomus carteri (Lowndes, 1934).	Ecotoxicology and environmental safety, (2020 Jun 15) Vol. 196, pp. 110501. Electronic Publication Date: 2 Apr 2020 Journal code: 7805381. E-ISSN: 1090-2414. L-ISSN: 0147-6513.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated.

Reference	Year	Title	Publication journal	Study overview and RMS justification
Garcia-Ruiz Esteban; Cobos Guillermo; Sanchez-Ramos Ismael; Pascual Susana; Chueca Maria-Cristina; Escorial Maria-Concepcion; Santin-Montanya Ines; Loureiro Inigo; Gonzalez-Nunez Manuel	2020	Dynamics of canopy-dwelling arthropods under different weed management options, including glyphosate, in conventional and genetically modified insect-resistant maize.	Insect science, (2020 May 27) . Electronic Publication Date: 27 May 2020 Journal code: 101266965. E-ISSN: 1744-7917. L-ISSN: 1672-9609.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated. Potentially relevant for indirect effects assessment.
Huaraca Luis F Chamorro Soledad A Hernandez Victor; Bay-Schmith Enrique Villamar Cristina A	2020	Comparative acute toxicity of glyphosate-based herbicide (GBH) to Daphnia magna, Tisbe longicornis, and Emerita analoga.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes, (2020) Vol. 55, No. 7, pp. 646-654. Electronic Publication Date: 20 May 2020 Journal code: 7607167. E-ISSN: 1532-4109. L-ISSN: 0360-1234.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated (despite that the composition of the tested formulations is not known).
Juginu, M. S.; Sujila, T.	2019	Histopathological effects of glyphosate in the gill, liver and kidney of the fresh water fish, Cyprinus carpio	International Journal of Advanced Research, (2019) Vol. 7, No. 10, pp. 907-914. CODEN: IJARND. ISSN: 2320-5407.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated.
Khan Sajida Zhou John L Ren Lei Mojiri Amin	2020	Effects of glyphosate on germination, photosynthesis and chloroplast morphology in tomato.	Chemosphere, (2020 Jun 11) Vol. 258, pp. 127350. Electronic Publication Date: 11 Jun 2020 Journal code: 0320657. E-ISSN: 1879-1298. L-ISSN: 0045-6535.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated.
Lanzarin Germano A B Venancio Carlos A S; Monteiro Sandra M Felix Luis M	2020	Behavioural toxicity of environmental relevant concentrations of a glyphosate commercial formulation - RoundUp® UltraMax - During zebrafish embryogenesis.	Chemosphere, (2020 Aug) Vol. 253, pp. 126636. Electronic Publication Date: 1 Apr 2020 Journal code: 0320657. E-ISSN: 1879-1298. L-ISSN: 0045-6535.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially

Reference	Year	Title	Publication journal	Study overview and RMS justification
				relevant parameters were investigated.
Maderthaner Michael; Weber Maureen; Querner Pascal; Walcher Ronnie; Gruber Edith; Zaller Johann G Takacs Eszter; Mortl Maria; Szekacs Andras Leisch Friedrich Rombke Jorg	2020	Commercial glyphosate-based herbicides effects on springtails (Collembola) differ from those of their respective active ingredients and vary with soil organic matter content.	Environmental science and pollution research international, (2020 May) Vol. 27, No. 14, pp. 17280-17289. Electronic Publication Date: 9 Mar 2020 Journal code: 9441769. E-ISSN: 1614-7499. L-ISSN: 0944-1344. Report No.: PMC-PMC7192858.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated.
Matozzo, Valerio ; Fabrello, Jacopo ; Marin, Maria Gabriella	2020	The Effects of Glyphosate and Its Commercial Formulations to Marine Invertebrates: A Review	Journal of Marine Science and Engineering, Vol. 8, No. 6, 20200101 E-ISSN: 2077-1312 DOI: 10.3390/jmse8060399 Published by: MDPI AG, Basel	No article is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title indicates that marine invertebrate species were investigated (that are relevant for the risk assessment). Such data may be used in a Weight of evidence assessment despite secondary source of information.
Nuutinen, Visa; Hagner, Marleena; Jalli, Heikki; Jauhiainen, Lauri; Ramo, Sari; Sarikka, Ilkka; Uusi-Kamppa, Jaana	2020	Glyphosate spraying and earthworm Lumbricus terrestris L. activity: Evaluating short-term impact in a glasshouse experiment simulating cereal post-harvest	European journal of soil biology (2020), Volume 96 ISSN: 1164-5563 Published by: Elsevier Masson SAS Source Note: 2020 Jan., Feb., v. 96	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated.
Sabio Y Garcia Carmen Alejandra Schiaffino Maria Romina Lozano Veronica Laura Vera Maria Solange; Izaguirre Irina; Pizarro Haydee Ferraro Marcela	2020	New findings on the effect of glyphosate on autotrophic and heterotrophic picoplankton structure: A microcosm approach.	Aquatic toxicology (Amsterdam, Netherlands), (2020 May) Vol. 222, pp. 105463. Electronic Publication Date: 3 Mar 2020 Journal code: 8500246. E-ISSN: 1879-1514. L-ISSN: 0166-445X.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated. Potentially relevant for indirect effects assessment.
Vazquez Diego E; Balbuena M Sol; Chaves	2020	Sleep in honey bees is affected by the herbicide glyphosate.	Scientific reports, (2020 Jun 29) Vol. 10, No. 1, pp. 10516. Electronic	No report is available for this study. It was identified by the

Reference	Year	Title	Publication journal	Study overview and RMS justification
Fidel; Farina Walter M Vazquez Diego E; Balbuena M Sol; Chaves Fidel; Farina Walter M Gora Jacob; Menzel Randolf			Publication Date: 29 Jun 2020 Journal code: 101563288. E-ISSN: 2045-2322. L-ISSN: 2045-2322. Report No.: PMC-PMC7324403.	applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated. Potentially relevant for the investigation of "other types of effects".
Whitlock, J. R. (Reprint); Vo, C. P.; Stahlschmidt, Z. R.	2020	Glyphosate in a warming world: Effects on lifespan, feeding, and food conversion efficiency in a field cricket, Gryllus lineaticeps	INTEGRATIVE AND COMPARATIVE BIOLOGY, (2020 MAR 2020) Vol. 60, pp. E444-E444. ISSN: 1540-7063.	No article is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title indicates that effects on field cricket were investigated (that are relevant for the risk assessment). Such data may be used in a Weight of evidence assessment despite lack of critical data.
Xiang Hongyong Xiang Hongyong Xiang Hongyong; Atkinson David Zhang Yixin Sekar Raju	2020	Effects of anthropogenic subsidy and glyphosate on macroinvertebrates in streams.	Environmental science and pollution research international, (2020 Jun) Vol. 27, No. 17, pp. 21939-21952. Electronic Publication Date: 13 Apr 2020 Journal code: 9441769. E-ISSN: 1614-7499. L-ISSN: 0944-1344.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated. Potentially relevant for indirect effects assessment.
Ximenes, Rodrigo Luiz; Gomes, Adelle Anik Araujo; Ximenes, Talia Simoes Dos Santos; Pires, Marta Siviero Guilherme	2020	Behavioral Analysis of Folsomia Candida (Collembola) with Herbicide Using Electronic and Computational Instrumentation: Bioassays	Soil & Sediment Contamination, (2020) Vol. 29, No. 5, pp. 545-556. CODEN: SSCOC6. ISSN: 1532-0383.	No report is available for this study. It was identified by the applicant as "non-relevant" in the rapid assessment. The title seems nevertheless to indicate that potentially relevant parameters were investigated.
Benamu M. A. et al.	2010	Effects of the herbicide glyphosate on biological attributes of Alpaida veniliae (Araneae, Araneidae), in laboratory	Chemosphere, (2010) Vol. 78, No. 7, pp. 871 6.	Study likely relevant as it addresses impact of glyphosate containing products on different stages of arthropods which hinders their capacity to persist in the agroecosystem.

Reference	Year	Title	Publication journal	Study overview and RMS justification
				A detailed summary should be provided together with an assessment of relevance and reliability.
Pleasants John M, Oberhauser Karen S	2013	Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population	Insect conservation and diversity (2013), Volume 6, Number 2, pp. 135-144 ISSN: 1752-458X Published by: Blackwell Publishing Ltd Source Note: 2013 Mar., v. 6, no. 2	Identified by RMS as potentially relevant for the risk assessment for NTA. Potentially relevant for indirect effects assessment (habitat loss). The study report, its summary and an assessment of relevance and reliability should be provided.
Boutin, Strandberg, Carpenter, Mathiassen, Thomas.	2014	Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications?	Environmental pollution (2014), Vol. 185, pp. 295-306	Identified by RMS as potentially relevant for the risk assessment for NTP (reproduction is considered relevant parameter despite not currently a data requirement). The study report, its summary and an assessment of relevance and reliability should be provided.
Carpenter D, Mathiassen SK, Boutin c, Strandberg B, Casey CS, Damgaard C.	2020	Effects of Herbicides on Flowering. Environ	Toxicol Chem 39(6):1244-1256. doi: 10.1002/etc.4712.	Identified by RMS as potentially relevant for the risk assessment for NTP (reproduction/flowering is considered relevant parameter despite not currently a data requirement). The study report, its summary and an assessment of relevance and reliability should be provided.
Cederlund H.	2017	Effects of spray drift of glyphosate on nontarget terrestrial plants-A critical review.	Environ Toxicol Chem. Nov;36(11):2879-2886. doi: 10.1002/etc.3925.	Identified by RMS as potentially relevant for the risk assessment for NTP. Such data may be used in a Weight of evidence assessment despite secondary source of information.

Reference	Year	Title	Publication journal	Study overview and RMS justification
				The study report, its summary and an assessment of relevance and reliability should be provided.
Damgaard C, Beate Strandberg, Solvejg K. Mathiassen & Per Kudsk	2014	The effect of glyphosate on the growth and competitive effect of perennial grass species in semi-natural grasslands	Journal of Environmental Science and Health, Part B, 49:12, 897-908, DOI: 10.1080/03601234.2014.951571	Identified by RMS as potentially relevant for the risk assessment for NTP. Potentially relevant for indirect effects assessment. The study report, its summary and an assessment of relevance and reliability should be provided.
Dupont, Strandberg, Damgaard	2018	Effects of herbicide and nitrogen fertilizer on non-target plant reproduction and indirect effects on pollination in Tanacetum vulgare (Asteraceae).	Agriculture, Ecosystems & Environment Volume 262, Pages 76-82	Identified by RMS as potentially relevant for the risk assessment for NTTP. Potentially relevant for indirect effects assessment. The study report, its summary and an assessment of relevance and reliability should be provided.
Schmitz, J., Schäfer, K., Brühl, C.A.,	2014	Agrochemicals in field margins — Field evaluation of plant reproduction effects.	Agric. Ecosyst. Environ. 189, 82–91. doi:10.1016/j.agee.2014.03.007	Identified by RMS as potentially relevant for the risk assessment for NTP (reproduction is considered relevant parameter despite not currently a data requirement). The study report, its summary and an assessment of relevance and reliability should be provided.
Strandberg, S.K. Mathiassen, M. Bruus, C. Kjaer, C. Damgaard, H.V. Andersen, R. Bossi, P. Løfstrøm, S.E. Larsen, J. Bak, P. Kudsk	2012	Effects of Herbicides on Non-target Plants: How Do Effects in Standard Plant Tests Relate to Effects in Natural Habitats?	Pesticide Research No 137 Danish Ministry of the Environment, EPA (2012), p. 114	Identified by RMS as potentially relevant for the risk assessment for NTP. Potentially relevant for biodiversity/indirect effects assessment. The study report, its summary and an assessment of relevance and reliability should be provided.

Reference	Year	Title	Publication journal	Study overview and RMS justification
Piola L., Fuchs J., Oneto M.L., Basack S., Kesten E., and Casabé N.	2013	Comparative toxicity of two glyphosate-based formulations to Eisenia andrei under laboratory conditions.	Chemosphere S0045-6535: 01537-8. doi: 10.1016/j.	Identified by RMS as potentially relevant for the risk assessment for earthworms. Potentially relevant for direct/indirect effects assessment. The study report, its summary and an assessment of relevance and reliability should be provided.

The Tables 9.11.1-2 (a. for first literature review and b. for the top-up literature review corresponding to documents KCA 9-001 and K-CA 9-002 of the applicant, respectively) to 9.11.1-3 listed the outcome of RMS analysis of the literature search after detailed assessment of full-text documents (from Literature Review Report of May 2020 and October 2020).

Table B.9.11.1.4-2.: Publications excluded from the risk assessment after detailed assessment of full-text documents

These tables correspond to the publications excluded, not proposed to be included in the assessment, belonging to category B or C or considered not relevant after full text. RMS updated the table by moving the studies considered relevant in the Table B.9.11.1-3 and Table B.9.11.1-4. RMS conclusion contained also studies identified after full text as data gap for further investigations.

Table B.9.11.1.4-2a.: From Literature Review Report KCA 9-001 (May 2020)

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Abalaka M. E. et al.	2015	Effects of pesticides on the micro- flora of loamy soil obtained from biological garden, federal university of technology, minna, Nigeria	Advance in Agriculture and Biology (2015), Vol. 4, No. 3, pp. 106-113	Presented data cannot be related to an EU level ANNEX I risk assessment (microbial population study).	The RMS does not agree with the applicant's reasoning and conclusion. Effect of pesticide application on soil microbial flora was studied. Bacterial and fungal population showed a decrease due to application of glyphosate (in formulation) to the soil samples. Some microorganisms like *Aspergillus* spp and *Bacillus subtilis* showed tolerance levels while others were suppressed. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Abalaka M. E. et al.2015.
Abdulkareem S. I. et al.	2013	Effects of sub-lethal concentrations of glyphosate on behaviour and some biochemical parameters of African catfish (Clarias gariepinus)	Proceedings of the 28th annual conference of the Fisheries Society of Nigeria (2013), pp. 188	5.4.1 case b) Relevant but supplementary information: Although blood, gill and liver enzyme levels are not relatable to an EU level ecotoxicological risk assessment the renewal purposes, the study was considered as supplemental due to the sublethal effects on fish behaviour following exposure to glyphosate. The study has not been conducted according to a recognised test guideline and there are no validity criteria presented. The fish	The RMS does not agree with the applicant's reasoning but nevertheless agrees that critical data are lacking. Behavioral observations are considered relevant by RMS. However as the fish loading may have had an impact on this parameter. RMS considers the results from this study not reliable.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				species and their origin are not described and environmental conditions (water quality) for the fish prior to and during the study have not been included. The fish loading rate (g/fish L test medium) was 20.5 g fish/L, which far exceeds the loading rate required for chronic static renewal fish tests typically required for studies submitted to support regulatory submission for renewals in the EU (0.8 to 1.0 g fish/L). The impact of such high fish densities cannot be established, as no water quality measurements were provided such as levels of dissolved oxygen (mgO2/L) and pH. Similarly, there was no test substance information or rationale presented for the selection of exposure concentrations. glyphosate concentrations were also not measured / confirmed during the 28 day study duration. Behavioural observations relating to the swimming activity are not relatable to the nominal exposure	
Abdulkareem S. I. et al.	2014	Effect of lethal and sub- lethal concentrations of glyphosate on some biochemical parameters and growth responses of African catfish (*Clarias gariepinus*).	Egyptian Academic Journal of Biological Sciences B Zoology (2014), Vol. 6, No. 2, pp. 47-54	Endpoints are not relatable to an EU level risk assessment. No information provided on the levels of exposure presented.	The RMS does not agree with the applicant's reasoning and conclusion. Parameters of relevance (for sublethal effects) seem to have been investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Abdulkareem S. I. et al.2014
Abdulkareem S. I. et al.	2015	Histopathological effects of lethal and sub- lethal concentrations of glyphosate on gills and liver of African catfish, Clarias gariepinus.	Journal of Aquatic Sciences (2015), Vol. 30, No. 1, pp. 53	5.4.1 case b) Relevant but supplementary information: Although blood, gill and liver enzyme levels are not relatable to an EU level ecotoxicological risk assessment for Annex I renewal purposes, the study was considering acute effects and chronic sublethal effects on fish following exposure to glyphosate. The study has not been conducted according to a recognised test	Behavioral observations and histopathological effects are considered relevant by RMS. However the fish loading may have had an impact on this parameter. The RMS agrees that critical data are lacking. RMS considers the results from this study not reliable.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				guideline and there are no validity criteria presented. The environmental holding conditions (water quality) for the fish prior to and during the study were not included. The fish loading rate (g/fish L) was 20.5 g fish/L, which far exceeds the loading rate required for chronic static renewal fish tests. The typical loading rates for studies submitted to support regulatory submission for Annex I renewals in the EU are 0.8 to 1.0 g fish/L. The impact of such high fish densities cannot be established, as no water quality measurements were included in ther paper, such as the dissolved oxygen levels (mgO2/L) and pH values. There was no test substance information presented, glyphosate concentrations were not measured / confirmed during the 28 day study duration. Behavioural observations in test vessels could not be related to the nominal exposure concentration. Finally, there were no quantifiable endpoints presented in the paper, considered applicable to an EU level ecotoxicological risk assessment for renewal purposes.	
Abraham J. et al.	2018	Commercially formulated glyphosate can kill non-target pollinator bees under laboratory conditions.	Entomologia Experimentalis et Applicata (2018), Vol. 166, No. 8, pp. 695-702	The study was conducted using Sunphosate 360 SL, which is not the representative formulation for the EU renewal at Annex I.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. This study investigates lethal effects on bee species and is thus relevant for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Abraham J. et al.2018
Achiorno C. L. et al.	2018	Susceptibility of *Chordodes nobilii* (Gordiida, Nematomorpha) to three pesticides: Influence of the water used for dilution on	Environmental pollution (2018), Vol. 242, No. Pt B, pp. 1427-1435	This paper describes the conduct of aquatic toxicity assays using naturally collected waters from the countries of interest. Infection rate of hosts was also assessed as an endpoint. Roundup that contains POEA was also	The presence of POEA in the tested formulation was not stated in the report. This study may then be considered as “less relevant but supplementary”.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		endpoints in an ecotoxicity bioassay.		used in the study. This surfactant is not in the representative formulation for the Annex I renewal.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Achiorno C. L. et al..2018
Ada F. B. et al.	2013	Ganado-hepato-somatic index of *Oreochromis niloticu*s sub adults exposed to some herbicides	International Journal of Aquaculture (2013), Vol. 3, No. 11	Endpoints based on gonadosomatic and hepatosomatic indeces are not used in the EU level ecotoxicological risk assessment for Annex I renewal.	The gonadosomatic and hepatosomatic indexes were measured after exposing the Nile Tilapia (Oreochromis niloticus) to Glyphosate (in Roundup) for 14 days. No information is available on the surfactants in the formulation that was used. Effects were observed but it is not possible to discriminate between glyphosate and surfactants. Atrophy in the two organs, liver and gonads were observed. No specific guideline was followed. No analytical verification available. Environmental conditions were not reported. Only graphs are presented. Statistics are unclear (absence of significance despite obvious decrease on hepatosomatic index). Overall the study is considered less relevant but supplementary (due to different formulation tested) and not reliable by RMS.
Afrifa A. A. et al.	2010	The effects of benomyl and glyphosate treated plant litter on nitrogen mineralization in mollisols.	West African Journal of Applied Ecology (2010), Vol. 17, pp. 143-152	In this study both glyphosate and a fungicide product are applied simultaneously to tomato plants. As this assesses combined effects this study is not relevant to the renewal of glyphosate.	Related to combined exposure of two compounds. Thus, RMS agrees with the applicant's reasoning and conclusion.
Agbon A. O. I. et al.	2014	The potential impact of Glyphosate on captured fisheries productivity and sustainability	Proceedings of the 29th annual conference of the Fisheries Society of Nigeria (2014), pp. 17	5.4.1 case b) Relevant but supplementary information: The study was not conducted according to a recognised acute test guideline and there are no validity criteria presented. The overall study duration was 35 days, from which a 96 hr LC50 value was determined. There are no data presented in the paper in terms of	Growth, haematological and biochemical parameters are considered relevant by RMS. However critical data are indeed lacking in this study (test item unknown, no analytical verification, conditions of the test). Thus RMS

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				mortalities over the first 4 days from which a 96 hr LC50 could be determined. The fish also appear to have been fed for the 35 day duration, which is not in accordance with the recognised acute fish toxicity test guideline used according to the EU No. 283 2013 data requirements. The environmental holding conditions (water quality) for the fish prior to and during the study were not included. The fish loading rate (g/fish L) cannot be determined as no test vessel water volumes are presented. There are no water quality measurements included in ther paper, such as the dissolved oxygen levels (mgO2/L) and pH values. There was no test substance information presented, glyphosate concentrations were not measured / confirmed during the 35 day study duration. No sub-lethal behavioural observations were included in the paper. Finally, the presented endpoints cannot be confirmed from the presented information in the paper. The study is considered unreliable.	considers the results from this study not reliable.
Agostini M. G. *et al.*	2020	Pesticides in the real world: The consequences of GMO-based intensive agriculture on native amphibians	Biological Conservation (2020), Vol. 241, Article ID 108355	Studies which can be difficult to extrapolate to EU (e.g. with local native species, geo-climatic properties, land-uses and agricultural practices, non-EU monitoring data, residue definitions differing from EU). Observations are caused by mixture of compounds / potentially causal factors and thus not attributable to a substance of concern (e.g. mixture toxicity - this paper looks at the impact of mixtures of pesticides rather than single actives). In addition, it is not clear which glyphosate-based herbicide was exactly tested.	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Aguilar-Dorantes K. et al.	2015	Glyphosate Susceptibility of Different Life Stages of Three Fern Species	American fern journal (2015), Vol. 105, No. 3, pp. 131	5.4.1 case b) Relevant but supplementary information: Considered supplementary as species not relateable to an EU level risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. Little is known on the sensitivity of fern species and the study may provide relevant data.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Aguilar-Dorantes K. et al..2015
Ahemad M. et al.	2012	Evaluation of plant-growth-promoting activities of rhizobacterium and *Pseudomonas putida* under herbicide stress	Annals of microbiology (2012), Vol. 62, No. 4, pp. 1531-1540	This paper discusses the impact of herbicides on the plant growth promoting activities of soil borne bacteria in the root zone. It is not relateable to an EU ecotoxicological risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Ahemad M. et al.2012
Akcha F. et al.	2012	Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos.	Aquatic toxicology (2012), Vol. 106-107, pp. 104-13	Endpoints derived from genotoxic screening and based upon parameters not considered relevant to EU renewal level assessment.	Genotoxicity is not a directly relevant parameter but still may be of interest in a Weight of evidence approach. Some parameters such as sperm viability were also investigated. Overall, no adverse effect of glyphosate was noted by the study authors. This study may be relevant for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Akcha F. et al.2012
Albajes R. et al.	2011	Two heteropteran predators in relation to weed management in herbicide- tolerant corn.	Biological Control (2011), Vol. 59, No. 1, pp.30-36	This study was not conducted to a relevant guideline. The test substance was identified as MON 78044, but no other test item information is provided (e.g. purity). The results of the study cannot clearly be related to the glyphosate treatments as multiple products were applied, the work is not GLP compliant and there is insufficient analytical documentation to confirm exposure.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Albajes R. et al.2011
Alcantara-de la Cruz R. et al.	2017	Side-effects of pesticides on the generalist endoparasitoid *Palmistichus*	Scientific Reports (2017), Vol. 7, No. 1, pp.10064	This paper discusses the influence of trait modified crops sprayed with glyphosate on biological control agents. It is not relateable to an EU level risk assessment.	The RMS agrees with the applicant’s reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		elaeisis (Hymenoptera: Eulophidae).			
Al-Daikh E. B. et al.	2016	Effect of glyphosate herbicide on the behavior of soil arthropods in non-organic tomato system	Advance in Agriculture and Biology (2016), Vol. 5, No. 1, pp. 14-19	Endpoints presented cannot be related to an EU level risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Al-Daikh E. B. et al.2016
Alhewairini S. S.	2017	Toxicity of the herbicide glyphosate to non- target species *Caenorhabditis elegans.*	Journal of Food, Agriculture & Environment (2017), Vol. 15, No. 2, pp. 97	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)	Caenorhabditis elegans is a 1mm nematode living in soil and feeding on bacteria. From RMS point of view this species is a model organism representing both nematodes and bacteria. Concentrations used are within the environmentally relevant concentrations. On these aspects the study should be further investigated for its appropriatness and relevance for risk assessment purpose. However there was no test substance information available. Exposure is expressed as glyphosate but the form used was unknown (IPA salt, K salt…) and it cannot be ascertain that commercial formulation (containing surfactants) was not used. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. Therefore RMS considers that this study is less relevant due to uncertainty on the test item. Besides RMS notes that dose-effect relationship shows an unusual trend.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					High mortality even at low concentrations were observed and a slow mortality increase between 1.2 ppm (40% mortality) and 1200 ppm (82% mortality) i.e. on a very large exposure range. No reliable endpoint can be derived. Assuming this species representative of a soil microorganism group, RMS notes that in other studies transitional effects of glyphosate were observed on microbial activity with recovery thereafter. This study does not inform on recovery rate. Concentrations at which feeding inhibition was affected (120 ppm and above) were above those expected for the representative uses intended. So no critical endpoint would be derived from this parameter. The study is considered not reliable for regulatory risk assessment purpose.
Alishahi M. et al.	2019	Comparative toxicities of five herbicides on nauplii of Artemia franciscana as an ecotoxicity bioindicator.	IRANIAN JOURNAL OF FISHERIES SCIENCES (2019), Vol. 18, No. 4, pp. 716	5.4.1 case b) Relevant but supplementary information: The material and methods section lack some important information. OECD standard methods were mentioned in the publication; however, the test guideline or specific validity criteria were not specified. Furthermore, information on preparation, application of the test item or exposure conditions are missing. No results for the control group are available to put the biological effects in context. Also no mortality results for all treatment group are given. At the end of the test, an endpoint was derived, but further statistical information (assessment of statistical power, confidence intervals) are not	RMS agrees with the applicant that no sufficient data is reported. Glyphosate showed significantly less lethality (higher LC50 concentration 164.31 mg/L) than other tested herbicides. RMS considers this study as : Less relevant but supplementary (due to uncertainty on the test item) and agrees to consider the study as not reliable.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				stated. Furthermore, there was no analytical verification of test concentrations reported. The study is considered unreliable.	
Alishahi M. et al.	2016	Acute toxicity evaluation of five herbicides: paraquat, 2,4-dichlorophenoxy acetic acid (2,4-D), trifluralin, glyphosite and atrazine in Luciobarbus esocinus fingerlings.	Iranian Journal of Veterinary Medicine (2016), Vol. 10, No. 4, pp. 319	5.4.1 case b) Relevant but supplementary information: Although the study was stated to have been conducted according to a recognised test guideline (OECD 203), no validity criteria was presented. The selected fish species and their approximate origin are described but environmental holding conditions (water quality) for the fish handling prior to and during the study were not included. There was limited test substance information presented, with no rationale presented for the selection of exposure concentrations. glyphosate concentrations were also not measured/confirmed during the evaluation period. Behavioural observations relating to the lethargy and swimming behaviour are not considered directly relatable to the nominal exposure concentration. The study is considered unreliable.	The 96 h LC50 of Glyphosate in *L.esocinus* was 716.83 mg/l. Glyphosate showed lowest toxicity in *Luciobarbus esocinus* among the five herbicides. RMS agrees with the applicant that no sufficient data is reported. Besides RMS notes the very high water total hardness (640 mg/l as CaCO3). RMS considers this study as : Less relevant but supplementary (due to uncertainty on the test item) and agrees to consider the study as not reliable.
Allegrini M. et al.	2015	Ecotoxicological assessment of soil microbial community tolerance to glyphosate.	The Science of the total environment (2015), Vol. 533, pp. 60-8	Novel test design / approach - not relatable to an EU level ecotoxicological risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Allegrini M. et al..2015
Allegrini M. et al.	2019	Suppression treatment differentially influences the microbial community and the occurrence of broad host range plasmids in the rhizosphere of the model cover crop Avena sativa L	PloS one (2019), Vol. 14, No. 10, pp. e0223600	Endpoints type is not considered at the EU level risk assessment and cannot be related to levels of exposure anticipated following application according to the proposed GAP.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Allegrini M. et al..2019
Allegrini M. et al.	2017	Repeated glyphosate exposure induces shifts in nitrifying communities and metabolism of phenylpropanoids	Soil biology & biochemistry (2017), Vol. 105, pp. 206-215	Approaches used cannot be related to an EU level ecotoxicological risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Allegrini M. et al. 2017
Alleva R. et al.	2016	Organic honey supplementation reverses pesticide-induced genotoxicity by modulating DNA damage response.	Molecular nutrition & food research (2016), Vol. 60, No. 10, pp. 2243-2255	Not related directly to the effects of glyphosate, but to the impact of polyphenols extracted from honey on human epithelial cells. Not relevant to EU level ecotoxicological risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Allison J. E. et al.	2013	Influence of soil organic matter on the sensitivity of selected wild and crop species to common herbicides.	Ecotoxicology ((2013), Vol. 22, No. 8, pp. 1289	5.4.1 case b) Relevant but supplementary information: Soils with a modified nutrient status were used which is not a requirement for the studies conducted to support the renewal in the EU.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. The high OM treatment was selected to roughly match OM levels in non-target areas of agroecosystems. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Allison J. E. et al.2013
Al-Sultany D. A. A. et al.	2019	Effects of contaminated water with glyphosate herbicides on the external and behavioral characteristics of common carp, *Cyprinus Carpio* Linnaeus.	Biochemical and Cellular Archives (2019), Vol. 19, No. 1, pp. 1475-1480	Methodology presented cannot be related to the results provided. Exposure rates cannot be related to the EU level assessment. No glyphosate formulation / product details presented.	The RMS agrees with the applicant's reasoning and conclusion. The report states that three concentrations were tested of 10%, 15% and 20%. These rates are indeed not relatable to the risk assessment. The study is not relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Alvarez M. et al.	2012	Toxicity in fishes of herbicides formulated with glyphosate	Acta Toxicologica Argentina (2012), Vol. 20, No. 1, pp. 5-13	Two formulations and a test solution prepared using technical material were used. The two formulations were glacoxan and Roundup. The Roundup contains POEA and therefore is not relevant to the EU. The Glacoxan is a home and garden use formulation that is not related to the representative formulation. It is therefore not relevant to an EU level risk assessment for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Alvarez M. et al. 2012
Amid C. et al.	2018	Additive effects of the herbicide glyphosate and elevated temperature on the branched coral *Acropora formosa* in Nha Trang, Vietnam.	Environmental science and pollution research international (2018), Vol. 25, No. 14, pp. 13360-13372	The paper discusses the combined impact of multiple stressors on coral bleaching, when exposed to a formulation that is not the representative formulation for the Annex I renewal. The study compares effects of the product on bleaching of corals at two different temperatures.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Amid C. et al.2018
Anbalagan C. et al.	2013	Use of transgenic GFP reporter strains of the nematode Caenorhabditis elegans to investigate the patterns of stress responses induced by pesticides and by organic extracts from agricultural soils.	Ecotoxicology (2013), Vol. 22, No. 1, pp. 72-85	Study provides information on cellular / molecular level and is not ecotoxicologically relevant study	RMS agrees that the study does not provide relevant data. Glyphosate only altered gene expression at very high concentrations. The study is not relevant.
Antoniolli Z. I. et al.	2013	Heavy metal, pesticides and fuels: effect in the population of collembola in the soil. Original Title: Metais pesados, agrotoxicos e combustiveis: efeito na populacao de colembolos no solo.	Ciencia Rural (2013), Vol. 43, No. 6, pp. 992-998	Concerns exposure to a glyphosate formulation (not the representative formulation) in the presence of metals, and in mixtures. It is not relevant to an EU level risk assessment.	Related to effects of combined exposure. Thus, RMS agrees with the applicant's reasoning and conclusion.
Antunes S. C. et al.	2010	Structural effects of the bioavailable fraction of pesticides in soil: suitability of elutriate testing.	Journal of hazardous materials (2010), Vol. 184, No. 1-3, pp. 215-25	The endpoint cannot be ascertained for glyphosate alone as other active substances are also used in the field study. The glyphosate product used (Montana) is not a representative product.	The RMS does not agree with the applicant's reasoning and conclusion. A replicated plot design (5 replicates per treatment) was used: pesticides were sprayed into 25 plots (control, where water was applied instead of

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					pesticide, plus four pesticide treatments) of 25m2 (5×5m) randomly distributed within the testing area and separated by 3 m-wide corridors in order to avoid cross-contamination. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Antunes S. C. et al..2010
Asgari S. M. et al.	2018	Oraganophosphorus pesticides induced enzymological responses in the various tissues of freshwater fish Koi carp (Cyprinus carpio)	European Journal of Zoological Research (2018), Vol. 6, No. 1, pp. 17-24	This study described the Biological impacts on enzyme levels in fish blood, are not used in an EU level ecotoxicological risk assessment.	The RMS agree with the applicant's reasoning and conclusion. Besides only high concentrations were tested.
Avigliano L. et al.	2014	Effects of glyphosate on egg incubation, larvae hatching, and ovarian rematuration in the estuarine crab *Neohelice granulata*	Environmental Toxicology and Chemistry (2014), Vol. 33, no. 8, pp. 1879	Article discusses effects of formulated product on crab development. Endpoints are not relatable to an EU level risk assessment as specific endpoints are not discussed.	This study was part of the previous EU assessment for endocrine disruption (2017). Ovigerous females of the estuarine crab (*Neohelice granulate*) were exposed to both pure glyphosate (2.5 mg/L and 5 mg/L) and a glyphosate formulation (Roundup Ultramax, containing glyphosate at 2.5 mg/L acid equivalent). This study investigated effects on developing embryos, hatched larvae, and ovarian rematuration of N. granulata during the reproductive period. The study did not follow standardized guideline. The nominal concentrations used for pure glyphosate were 2.5 mg/L and 5 mg/L, whereas 2.5 mg/L was used for the Roundup Ultramax treatment.

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					Analytical verifications of tested concentrations have been made. No mortality or effect on bodyweight was observed on ovigerous females. Because no loss of eggs was verified in any case, 100% of females had normal hatching. A significant reduction in the number of hatched larvae per female was observed in the Roundup treatment. (note: exposure period till hatching 9.86 d). A significantly higher incidence of both hydropsy and hypopigmented eyes was observed for both glyphosate and Roundup groups (2.5 mg/L of glyphosate a.e. in both cases). Pure glyphosate at 2.5 mg/L stimulated ovarian maturation, mainly in terms of a higher gonadosomatic index and a higher percentage of vitellogenic oocytes (after 32 days exposure). A significantly lower number of hatched larvae per female was detected in the Roundup Ultramax treatment, a clear embryonic mortality was associated with this formulation, which contained a glyphosate concentration of 2.5 mg/L. Also taking into account that pure glyphosate, at the same concentration, did not significantly reduce the number of hatched larvae, these results indicate that Roundup compounds other than glyphosate may be responsible for the embryonic mortality.

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					The results available for the pure form of glyphosate are deemed relevant by RMS (i.e. stimulated ovarian maturation at 2.5 mg/L, mainly in terms of a higher gonadosomatic index and a higher percentage of vitellogenic oocytes). This study shows effects potentially related to endocrine disruption. In accordance with the EFSA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, such effects (including malformations) should be considered. Relevant for the assessment of potential for endocrine disruption. The results of the range-finding study were not presented. Then the MTC cannot be determined. Concerning the potential impact of using wild-caught organisms, particularly regarding potential effects on the endocrine system from prior exposure to other substances, it is mentioned in the report that the specimens were randomly collected at the southern edge of Samborombón Bay, a "nonpolluted" area at the mouth of the Rio de la Plata estuary, Argentina. The contamination level cannot be verified by RMS, it is only stated that very scarce information about glyphosate environmental

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					levels has been published, with reported values between 0.1 mg/L and 0.7 mg/L in water and between 0.5 mg/kg and 5mg/kg in sediments. The study authors then assume the tested concentrations are higher than the overall levels of glyphosate found in Argentina but still relevant for a direct run-off entry and comparable to levels measured in other countries. Besides, Avigliano et al, 2018 (also assessed by RMS) states that "heavy charge of herbicides and other pesticides is carried by several rivers and channels that cross extensive agricultural areas and finally reach the Samborombón Bay (Comisión Administrativa del Río de La Plata 1990)". RMS then cannot discard the presence of other toxicants in this estuary. Control mortality was relatively high at the end of the assay (27% at 32 days) although RMS acknowledges that data on "natural" mortality is lacking. Mortality averaged 9% in the glyphosate treatments, which did not differ from control. Regarding the morphology of the larvae, statistically significant effects were observed in the treatment with lower glyphosate, but not in the treatment with higher glyphosate concentrations. In the glyphosate treatments, data showed high variability, which likely hampered the detection of statistically significant results. The observed increases in gonadosomatic index (without

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					concurrent hepatosomatic index increases) are likely due, as the authors supposed, to increased egg resorption, but the reason or the specific mechanism for these observation remains unclear and might be due to general toxicity. It is noted that the effects on morphology did not follow a dose-response relationship in the treatments with glyphosate. Overall, it is not possible to relate the observed effects to an endocrine mode of action. The study is relevant but results considered unreliable for ED assessment.
Ayanda I. O. et al.	2018	Toxicity of sublethal concentrations of glyphosate and paraquat herbicide in the African catfish (*Clarias gariepinus*)	International Journal of Agriculture and Biology (2018), Vol. 20, No. 6, pp. 1359-1364	Observations based on enzyme levels are not used in EU level ecotoxicological risk assessment for Annex I renewal purposes.	The RMS agree with the applicant's reasoning and conclusion.
Ayanda O. I. et al.	2015	Acute toxicity of glyphosate and paraquat to the African catfish (Clarias gariepinus, Teugels 1986) using some biochemical indicators	Tropical zoology (2015), Vol. 28, No. 4, pp. 152	5.4.1 case b) Relevant but supplementary information: The test item was not identified, therefore it is not clear what was actually tested and to which compound the effects / results can be assigned. The study is considered unreliable.	The relevance of these biochemical indicators for the risk assessment cannot be established by RMS as no quantitative link can be made between these parameters and the potential adverse effect at population level (this latter being the specific protection goal). So only results on mortality were considered by RMS. The present study shows that acute concentrations of glyphosate are toxic to African catfish Clarias gariepinus. After 96 h of exposure, the 96 h LC50

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					for glyphosate was found to be 0.530 mg/L. LOEL mortality (and biochemical effects) = 0.36 mg/L, with 16.67% mortality. However there was no test item detailed information available. Exposure is expressed as glyphosate but the form is unknown. Moreover, it cannot be ascertain that commercial formulation (containing surfactants) was not used (test item purchased from a commercial outlet). Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. Because of uncertainty on test item, the study is considered less relevant but supplementary by RMS. The OECD 203 gave recommendations for the acclimatisation phase indicated that if mortalities are greater than 10% during the first seven days, the batch should be rejected. Here, it was indicated that less than 5% mortalities was obtained after 14 days. There is no indication of the initial sensitivity of the fish to laboratory conditions. This may affect the outcome of the test. No analytical verification is available. Dose effect relationship was observed indicating that dosing was somehow adequate, nevertheless uncertainty remains on the actual concentrations.

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					In absence of clear identification of the product used and its similarity with the current EU representative formulation, RMS considers that this study is of low reliability and cannot be taken into account as a reliable information for the assessment of the active substance glyphosate itself. Thus, RMS agrees to classify the study as less relevant but supplementary and not reliable for risk assessment purpose after detailed assessment of full-text article.
Babalola O. O. *et al.*	2019	Mortality, teratogenicity and growth inhibition of three glyphosate formulations using Frog Embryo Teratogenesis Assay-*Xenopus*.	Journal of applied toxicology (2019), Vol. 39, No. 9, pp. 1257-1266.	This paper uses a formulation that is not the representative formulation for the annex I renewal. Study endpoints cannot be related to the EU level risk assessment as the techniques used are not recognised at the EU level.	The RMS does not agree with the applicant's reasoning and conclusion. The results on the Enviro and Kilo Max formulation are considered relevant and less relevant but supplementary, respectively. The study is considered reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Baglan H. et al.	2018	Glyphosate impairs learning in Aedes aegypti mosquito larvae at field-realistic doses.	The Journal of experimental biology (2018), Vol. 221, No. 20, pp 1	5.4.1 case b) Relevant but supplementary information: Information presented on the learning behaviour of mosquito larvae exposed to glyphosate. These data are difficult to relate to an EU level ecotox risk assessment for the renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information to be used in a weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Baglan H. et al.2018
Balbuena M. S. et al.	2015	Effects of sublethal doses of glyphosate on honeybee navigation.	The Journal of experimental biology	5.4.1 case b) Relevant but supplementary information.	In this study homeward trajectories of honeybees were tracked using harmonic radar technology.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
			(2015), Vol. 218, No. 17, pp. 2799		Honeybees that had been fed with solution containing 10 mg.l−1 glyphosate spent more time performing homeward flights than control bees or bees treated with lower concentrations. They also performed more indirect homing flights. The proportion of direct homeward flights performed after a second release from the same site increased in control bees but not in treated bees. These results suggest that, in honeybees, exposure to glyphosate impairs the cognitive capacities needed to retrieve and integrate spatial information for a successful return to the hive. LOEL = 10 mg glyphosate/L in sucrose solution. RMS notes the absence of clear conceptual link between flight time and the specific protection goals for bees (SPG). It is agreed that a longer duration of foraging trips may play a role in the colony/population health, but such link is not immediate in conceptual terms and not quantifiable. The results of this study are not directly relevant for risk assessment purpose but still relevant to address "other effects". Reliability was not assessed in depth but results are considered unreliable. The study states that bees fed with solution containing 10 mg.l−1 glyphosate spent more time performing homeward flights than control bees or bees treated with lower concentrations and also performed

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					more indirect homing flights. However the number of bees used in the experiments were very low and not sufficient to represent the natural variability of the parameters investigated (about 10 individuals at the dose of 10 mg/L or even less). Overall, the study is relevant but not reliable.
Banaee M. et al.	2019	Acute exposure to chlorpyrifos and glyphosate induces changes in hemolymph biochemical parameters in the crayfish, *Astacus leptodactylus* (Eschscholtz, 1823).	Comparative biochemistry and physiology. Toxicology & pharmacology (2019), Vol. 222, pp. 145-155	Endpoints based on biochemical parameters are not relatable to an EU level ecotoxicological risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. Relevant parameters such as mortality were investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Banaee M. et al.2019
Bandara K. et al.	2015	Effect of glyphosate-based herbicide, Roundup super (TM) on territory deference of male Oreochromis mossambicus (Osteichthyes, Cichlidae) associated with mating behaviour	Sri Lanka journal of aquatic sciences (2015), Vol. 20, No. 1, pp. 1-10	Formulation tested contains POEA - not relevant to an EU level Annex I ecotoxicological risk assessment for renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Bara J. J. et al.	2014	Sublethal effects of atrazine and glyphosate on life history traits of Aedes aegypti and Aedes albopictus (Diptera: Culicidae).	Parasitology research (2014), Vol. 113, No. 8, pp. 2879	5.4.1 case b) Relevant but supplementary information: The test provides information on the impact of glyphosate on mosquito development, but the test design employed is not a recognised approach used for Annex I data generation for renewal purposes. Test item purity not stated, only pestanol grade. No chemical analysis.	The RMS agrees with the applicant's reasoning and conclusion and notes that adverse effects were only found for atrazine and not glyphosate (at nominal concentration 5 mg/L). The study does not provide relevant endpoint for the risk assessment.
Barbukho O. V. et al.	2011	Effect of herbicide Roundup on carp spawn viability and possibility for prevention of its toxicity by probiotic preparation BPS-44	Gidrobiologicheskii Zhurnal (2011), Vol. 47, No. 3, pp. 74-79	As Roundup was used in the study which contains surfactants not present in the representative formulation high concentrations were used, and eggs were exposed to both a probiotic and Roundup, this study is not relevant to the renewal of glyphosate.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Not only high concentrations were tested.

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					Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Barbukho O. V. et al..2011.
Barriuso J. et al.	2011	Effect of the herbicide glyphosate on the culturable fraction of glyphosate-tolerant maize rhizobacterial communities using two different growth media.	Microbes and environments (2011), Vol. 26, No. 4, pp. 332	5.4.1 case b) Relevant but supplementary information: The study was a comparison between glyphosate and Harness GTZ (pre-emergence herbicide). glyphosate (Roundup plus) was applied at appropriate concentrations (360 g/kl, 0.72 kg/ha), the study looked at the rhizobacterial communities of glyphosate tolerant maize. The study was not to any relevant guideline and did not provide an endpoint relevant to the renewal of glyphosate.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Barriuso J. et al...2011
Bawa V. et al.	2018	Toxic effects of glyphosate on common carp (Cyprinus carpio L.) fingerlings.	Agricultural Research Journal (2018), Vol. 55, No. 1, pp. 169-171	The formulation used has a surfactant system that is based on POEA, which is not relevant to the EU representative formulation for the annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Behrend J. E. et al.	2018	Contact with a glyphosate-based herbicide has long-term effects on the activity and foraging of an agrobiont wolf spider.	Chemosphere (2018), Vol. 194, pp. 714-721	Study used MON 8709 Buccaneer Plus formulation which contains MON 0818 (based on POEA) and is not used in the representative EU formulation. Therefore findings cannot be related to the risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Berger G. *et al.*	2018	How does changing pesticide usage over time affect migrating amphibians: a case study on the use of glyphosate-based herbicides in German agriculture over 20 years.	Frontiers in Environmental Science (2018), Vol. 6, article 6	This is country-specific information that cannot be related to an EU level ecotoxicological regulatory risk assessment for EU Annex I renewal. Moreover, no formulations tested were specifically mentioned.	The RMS agrees with the applicant's conclusion that this study is not relevant for the risk assessment of MON 52276 for this evaluation, since there were no specific results on toxicity of glyphosate. Possibly, the results may be useful for discussion on risk mitigation options.
Bernal-Rey D. L. et al.	2020	Seasonal variations in the dose-response relationship of acetylcholinesterase activity in freshwater fish exposed to chlorpyrifos and glyphosate.	Ecotoxicology and environmental safety (2020), Vol. 187, pp. 109673	No specific endpoints that are useable in an EU level ecotoxicological risk assessment for Annex I renewal are presented in the paper. It is difficult to relate the observed effects to fish species found in the EU, as these data were collected from wild caught fish collected over a period of time.	The RMS agrees with the applicant's reasoning and conclusion. This study does not provide data relatable to the risk assessment.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				Impacts for example, of pH on the levels of stress in the system were not considered and may have ultimately contributed to the observed effects.	
Berthelemy N. J.	2018	Effects of Glyphosate and Roundup on the brine shrimp *Artemia franciscana*	Integrative and comparative biology (2018), Vol. 58, Supp. 1, pp. E277-E277	This paper is a poster abstract. There is no associated paper. There is insufficient information presented in the poster abstract to establish relevance of the poster to the Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Blasco P. M. P. et al.	2018	Comparative study of chronic toxicity of herbicides used in South America using a model of *Cyprinus carpio*	Environmental Science: An Indian Journal (2018), Vol. 14, No. 5, pp. 175	Formulation used is not the representative formulation for the Annex I renewal.	Not relevant for the assessment of ED effects: When assessing the potential impact of glyphosate on the endocrine system in fish, this study is not considered relevant since no ED related parameters are investigated.
Blot N. et al.	2019	Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota	PloS one (2019), Vol. 14, No. 4, pp. e0215466	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. In this publication, experiments were conducted with a dose (10x increased) and an exposure for a longer period than is expected to occur from field exposure. Results indicated no effect on survival but some effect on profile of gut microbes. AMPA did not affect profile which could be due to AMPA does not inhibit EPSPS.	Results indicated that glyphosate had some effect on honeybee microbiota but not AMPA. The present study states that its results confirm those of Motta et al. 2018 by showing that glyphosate, although not lethal for the honeybee, can alter its gut microbiota. (Motta et al. 2018 was also assessed by RMS.) It states that "in relative abundance, a strong decrease in of S. alvi and an increase in Lactobacillus spp. were observed in response to glyphosate, which was consistent with the data of Motta et al. 2018 in newly emerged and interior bees. In contrast, opposite results were observed on G. apicola. In absolute abundance, Motta et al. 2018 showed that glyphosate induced a reduction in the total bacterial content of the honeybee gut microbiota, as well as a decrease in S. alvi, Lactobacillus spp. and Bifidobacterium spp.".

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Several experiments were conducted in the study to show that exposure of adult bees might impact the gut microbiome leading to a greater susceptibility to pathogen infections. On this aspect, the study is not directly relevant for the risk assessment but still relevant for the investigation of "other types of effects" not currently covered by the risk assessment scheme. It is considered by RMS as "additionnal data". RMS notes the absence of clear conceptual link between effects on the honeybee microbiota and the specific protection goals for bees (SPG). It is agreed that it may play a role in the colony/population health, but such link is not immediate in conceptual terms and not quantifiable. No data can be used for the standard risk assessment. However only graphics are reported, no biological data reported. Besides, as noted for Motta et al, 2018, some shortcomings render the study lowly reliable (e.g. small sample sizes, influence of age on gut microbiome, lack of confirmation of the levels of actual glyphosate exposure, bees diets had no source of amino acids, etc.). Overall, the study is considered relevant but not reliable.
Boily M. et al.	2013	Acetylcholinesterase in honey bees (Apis mellifera) exposed to neonicotinoids, atrazine and	Environmental science and pollution research international (2013),	The test item is the commercial formulation Weathermax 240 which is distributed in Canada. This formulation is not the representative formulation for the Annex I renewal in the EU. In	This study does not provide data relatable to the risk assessment. RMS considers this study not relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		glyphosate: laboratory and field experiments.	Vol. 20, No. 8, pp. 5603-14	addition, the study does not follow any approved guideline and the investigated effect on acetylcholinesterase cannot be related to the EU level bee ecotoxicological risk assessment for Annex I renewal purposes. The field experiment, conducted in two regions in Québec (Canada) was not conducted under controlled conditions. No analytical verification of glyphosate was provided. Also, the experimental design is only briefly described, with no rationale presented for the selection of exposure concentrations.	
Bokony V. et al.	2017	Chronic exposure to a glyphosate-based herbicide makes toad larvae more toxic.	Proceedings. Biological sciences (2017), Vol. 284, No. 1858	The article does not report results which can be used for a risk assessment and information is insufficient to transfer values into such determinants.	The RMS agrees with the applicant's reasoning and conclusion.
Bonnineau C. et al.	2012	Light history modulates antioxidant and photosynthetic responses of biofilms to both natural (light) and chemical (herbicides) stressors.	Ecotoxicology (2012), Vol. 21, No. 4, pp. 1208-24	Endpoints / findings not relatable to an EU level ecotoxicolgical risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Boonsoong B. et al.	2012	Acute toxicity of Roundup and carbosulfan to the Thai fairy shrimp, Branchinella thailandensis.	Communications in agricultural and applied biological sciences (2012), Vol. 77, No. 4, pp. 431	5.4.1 case b) Relevant but supplementary information: The study was not conducted according to a recognised test guideline and no validity criteria are presented for control group performance, so the robustness of the assay can not be concluded. In the materials and methods, there is insufficient information presented on the test medium preparation approach and on the environmental conditions used in the test. There was no chemical analysis and therefore exposure cannot be confirmed. There are insufficient explanations provided on the experimental design, particularly environmental condition and conduct during the test. The study is considered unreliable.	The RMS agrees with the applicant's reasoning and conclusion. It was hypothesized that the high toxicity of Roundup was due to POEA. Overall, the study is not reliable.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Bortoli P. V. et al.	2012	Effects of glyphosate on microbial community structure and activity in two soils under olive plantations. Original Title: Efectos del herbicida glifosato sobre la estructura y el funcionamiento de comunidades microbianas de dos suelos de plantaciones de olivo.	Ecologia Austral (2012), Vol. 22, No. 1, pp. 33	5.4.1 case b) Relevant but supplementary information: Paper presents information on the effects of glyphosate on respiration but the approaches used do not result in endpoints that can be used in an EU level risk assessment as they are based on Argentinian soils.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Bortoli P. V. et al.2012
Boscardin J. et al.	2016	Effects of different types of weed control on the ant fauna in *Eucalyptus grandis*. Original Title: Efeitos de diferentes tipos de controle de plantas infestantes sobre a mirmecofauna em Eucalyptus grandis.	Ciencia Florestal (2016), Vol. 26, No. 1, pp. 21-34	Specific endpoints that could be used in an EU level risk assessment were not presented.	The RMS agrees with the applicant's reasoning and conclusion.
Boscardin J. et al.	2014	Relationship between ant communities and environmental quality in Eucalyptus grandis submitted to different weedy species control in the south of Brazil. Original Title: Relacao entre guildas de formigas e a qualidade ambiental em Eucalyptus grandis subme	ENTOMOTROPICA (2014), Vol. 29, No. 3, pp.173-182	Presented data is not relatable to an EU level risk assessment for EU Annex I level renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Bott S. et al.	2011	Phytotoxicity of glyphosate soil residues re- mobilised by phosphate fertilisation	Plant and soil (2011), Vol. 342, No. 1-2, pp. 249	5.4.1 case b) Relevant but supplementary information: Roundup ultra max (360 g/L, applied up to 4.8 mg ae/kg soil), study looked at the impact of phosphate and glyphosate competition in the soil and subsequent availability of NTTP and impact on soil characteristics (in different soil types) to soybean growth. AMPA is also considered in the article. However, a regulatory endpoint suitable for the	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Bott S. et al. 2011

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				renewal of glyphosate was not obtained from the article.	
Boufleuer E. M. S. et al.	2016	Assessment of mortality and reproduction of *Daphnia magna* subjected to the herbicide glyphosate. Avaliacao da mortalidade e reproducao de Daphnia magna submetida ao herbicida glifosato.	Acta Iguazu (2016), Vol. 5, No. 5, pp. 25-33	Results of a 48 hour Daphnia magna tests treated with glyphosate determined an LC50 of 2.1087 mg/L. A chronic (21 day) study determined effects at 2.1087 mg/L, but no effects were observed at the lower concentrations tested. The study was not conducted to GLP or to an acceptable guideline and there are several short comings in the provided report. The test substance used (Polaris 48%) is a Monsanto Brazil product that is based on the IPA salt of Glyphosate. This product also contains a surfactant that is not relevant to the representative formulation, therefore the observed findings are not considered relevant to the renewal. Furthermore the influence of the co-formulant on the results cannot be excluded. There are no analytical data reported and so the exposure cannot be confirmed.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Boufleuer E. M. S. et al. 2016
Boutin C. et al.	2010	Measuring variability in phytotoxicity testing using crop and wild plant species.	Environmental toxicology and chemistry (2010), Vol. 29, No. 2, pp. 327-37	Glyphosate product + surfactant (Agral 90) was used in the study which compared this with an atrazine product to look at the phytotoxicity to plant species. Treatments ranged from 21 to 2277 g ai/ha for glyphosate product, applied in a greenhouse. Although an IC25 could be obtained from the article, the results indicate great variability between the plant species tested and external factors. Therefore, it is not possible to extrapolate from this data for use in the regulatory risk assessment in the glyphosate renewal.	The RMS does not agree with the applicant's reasoning and conclusion. It was shown that test conditions induced a large variability (in a given species) in response to herbicides. Both crops and wild plant species responded quite variably when they were tested in different seasons as well as when tested in a greenhouse or in growth chambers. The present study supports the inclusion of an uncertainty factor in risk assessments to account for the intrinsic variability in plant sensitivity to herbicides. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Boutin C. et al. 2010

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Boutin C. et al.	2019	Effects of sub-lethal doses of herbicides on the competitive interactions between two non-target plants: Centaurea cyanus L. and Silene noctiflora L.	Environmental toxicology and chemistry (2019), Vol . 8, No. 9, pp. 2053-2064	Observation not linked to glyphosate or its metabolites. In this case the observations were concerning competition in the growth of plants under different pesticide stress regimes and at different planting densities. Endpoints considered relevant for EU level risk assessment were not presented.	The RMS agrees with the applicant's reasoning and conclusion.
Bridi D. et al.	2017	Glyphosate and Roundup(®) alter morphology and behavior in zebrafish.	Toxicology (20171), Vol. 392, pp. 32-39	The article does not report results, which can be used for risk assessment and information is insufficient to transfer values into such determinants.	The aim of this study was evaluate the effects of glyphosate and Roundup® on behavioral and morphological parameters in zebrafish larvae and adults. Immediately after the exposure, locomotor activity, aversive behavior, and morphology for the larvae and exploratory behavior, aggression and inhibitory avoidance memory for adult zebrafish were investigated. These parameters are deemed relevant by RMS. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Bridi D. et al. 2017
Bruckner A. et al.	2019	Foliar Roundup application has minor effects on the compositional and functional diversity of soil microorganisms in a short-term greenhouse experiment.	Ecotoxicology and environmental safety (2019), Vol. 174, pp. 506-513	Formulation used is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Bruckner A. et al. 2019
Buch A. C. et al.	2013	Toxicity of three pesticides commonly used in Brazil to Pontoscolex corethrurus (Mueller, 1857) and Eisenia andrei (Bouche, 1972)	Applied soil ecology (2013), Vol. 69, pp. 32-38	The formulation used is not the representative formulation for the Annex I.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Buch A. C. et al. 2013
Canosa I. S. et al.	2018	Ovarian growth impairment after chronic exposure to Roundup Ultramax® in the estuarine crab Neohelice granulata.	Environmental science and pollution research international (2018), Vol. 25, No. 2, pp. 1568-1575	Roundup Ultramax is the formulation used which contains 600 g/L a.e. This is however, not the representative formulation for the renewal.	This study originates from the same lab that produced the following study: Avigliano L. et al., 2014; Effects of glyphosate on egg incubation, larvae hatching, and ovarian rematuration in the estuarine crab Neohelice granulate. Avigliano L. et al., 2014 stated that based on a comparison of number of hatched larvae per female between Roundup Ultramax (clear embryonic mortality) and glyphosate (no significant increase of mortality) at equivalent concentration, Roundup compounds other than glyphosate may be responsible for the embryonic mortality. RMS then doubts the relevance of the data in this study (Canosa I. S. et al., 2018) as only Roundup Ultramax was used and no discrimination between glyphosate and other compounds is feasible. The study is considered not relevant.
Carmo E. L. et al.	2010	Pesticide selectivity for the insect egg parasitoid Telenomus remus	BioControl (2010), Vol. 55, No. 4, pp. 455-464	An IOBC guideline criteria was used for classification of three different glyphosate products used as test substances alongside several other insecticides and herbicides in this comparison lab study. Endpoints generated are not relevant to the renewal of glyphosate.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information to be used in a weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					for the paper of Carmo E. L. et al. 2010
Carranza C. S. et al.	2014	Influence of the pesticides glyphosate, chlorpyrifos and atrazine on growth parameters of nonochratoxigenic Aspergillus section Nigri strains isolated from agricultural soils.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2014), Vol. 49, No. 10, pp. 747-55	Comparative growth rates of Apsergillus niger following application of different pesticides. Endpoints are not relatable to an EU level Annex I ecotoxicological risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Carvalho L. B. et al.	2016	Plant Growth Responses of Apple and Pear Trees to Doses of Glyphosate	Planta Daninha (2016), Vol. 34, No. 4, pp. 815	5.4.1 case b) Relevant but supplementary information: Study investigates the impact of spraying apple and pear saplings at rates up to 720 g/ha and assesses effects on yield. Spraying of sapling trees directly is not on the GAP table as a use, so whilst they may inform on the potential risk via drift, endpoint considered relevant to EU level risk assessment. The endpoints were not established using a test guideline considered relevant to EU renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information on woody species. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Carvalho L. B. et al. 2016
Castilho A. F. et al.	2016	The impact of glyphosate herbicides on soil microbial activity from the Carajas National Forest.	Revista de Ciencias Agrarias / Amazonian Journal of Agricultural and Environmental Sciences (2016), Vol. 59, No. 3, pp. 302-309	A long term monitoring study using multiple Roundup formulations was performed. Roundup original contains POEA as a surfactant and is not therefore relevant. The other Roundup formulations differ in their composition to the representative formulation for the Annex I renewal.	From other Sanchez et al 2017 that states « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS. For other formulation, the study could be considered as less relevant but supplementary and reliability should be assessed.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a summary and assessment of relevance and reliability for Castilho A. F. et al.2016.
Castilhos R. V. et al.	2011	Selectivity of pesticides used in peach orchard on adults of Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae). Original title: Seletividade de agrotoxicos utilizados em pomares de pessego a adultos do predador Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae).	Revista Brasileira de Fruticultura (2011), Vol. 33, No. 1, pp. 73	5.4.1 case b) Relevant but supplementary information: Roundup (and many other pesticides) were used as the test substance. Only mortality of lacewing were assessed. Likewise no reproduction endpoints were evaluated and thus no data is relevant to the risk assessment.	The RMS agrees with the applicant's reasoning and conclusion as glyphosate was found harmless to adults of Chrysoperla externa (based on mortality). Then no relevant information would be obtained from this study.
Castilhos R. V. et al.	2014	Selectivity of pesticides used in peach orchards on eggs and pupae of the predator Chrysoperla externa. Seletividade de agrotoxicos utilizados em pessegueiro sobre ovos e pupas do predador Chrysoperla externa.	Ciencia Rural (2014), Vol. 44, No. 11, pp. 1921	5.4.1 case b) Relevant but supplementary information: The glyphosate product was concluded to be harmless to Chrysoperla and Chrysoperla eggs and pupae. The study was not conducted according to GLP and the study design lacks some details compared with relevant guidelines. The test concentrations are based on nominal values and no analytical verification of test item concentrations was conducted. Although the test design is described in quite some detail, some important information is missing, i.e. regarding the source and content of the applied products, the application of test item and control data are not shown for all parameters. Additionally, according to IOBC/WPRC larval stages should be exposed. As the study is based on a glyphosate product, the toxicity of glyphosate active substance alone is unknown and therefore endpoints generated from this study are not quantifiable and deliver only supplementary information.	The RMS agrees with the applicant's reasoning and conclusion as glyphosate was found harmless to eggs and pupae of the predator Chrysoperla externa. Then no relevant information would be obtained from this study.
Cattaneo R. et al.	2011	Toxicological responses of Cyprinus carpio exposed to a	Bulletin of environmental contamination and	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)	Due to presence of POEA, the study is not relevant by RMS for assessment of the current EU representative

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		commercial formulation containing glyphosate.	toxicology (2011), Vol. 87, No. 6, pp. 597		formulation MON52276. The reliability of the study was not assessed.
Cavusoglu K. et al.	2011	Investigation of toxic effects of the glyphosate on Allium cepa.	Tarim Bilimleri Dergisi (2011), Vol. 17, No. 2, pp. 131	5.4.1 case b) Relevant but supplementary information: Glyphosate products were used in the study. Impact on seed germination and root growth.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Cavusoglu K. et al. 2011
Chandrasekera W. U. et al.	2011	The lethal impacts of Roundup® (glyphosate) on the fingerlings of guppy, Poecilia reticulata Peters, 1859.	Asian Fisheries Science (2011), Vol. 24, No. 4, pp. 367	5.4.1 case b) Relevant but supplementary information: The material and methods lacks important information. The purity of the formulation is not presented. There is a narrative on water qualities / environmental conditions during the test, but there is no actual data presented to confirm the acceptability of the exposure / test conditions except for a value presented for dissolved oxygen levels. There was no analytical verification of test concentrations reported and therefore the level of exposure cannot be confirmed. The study is considered unreliable.	The lethal effects of commercial glyphosate formulation Roundup were studied on fingerlings of guppies (Poecilia reticulata). Further, the behavioural changes of the fingerlings and the histopathological changes of their gills were examined. In view of the information reported in the article and its year of publication, it is very likely that the surfactant polyethoxylated tallow amine (POEA) was used in the formulation, which is known to be more toxic than glyphosate to fish. Besides, to justify the choice of the test item, the authors report in the introduction that "although acute toxicity of glyphosate itself is considered to be low, commercial glyphosate formulations such as Roundup® are more toxic as they contain a surfactant called poly-oxy ethylene amine. This surfactant is used to promote penetration of glyphosate through plant cuticle thereby enhancing its efficiency".

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Since August 2016, POEA is not authorized in plant protection products containing glyphosate (European Commission). Effects (lethal and sublethal) were observed but it is not possible to discriminate between glyphosate and POEA. Due to potential presence of POEA, the study is considered not reliable relevant by RMS for assessment of the current EU representative formulation MON52276. The reliability of the study was not assessed.
Chen L. et al.	2012	The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria.	Ecotoxicology and environmental safety (2012), Vol. 80, pp. 224-30	Paper discusses the effect of glyphosate at different UV-B levels. Direct effects are not discussed. Not relatable to EU level risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Choi C. J. et al.	2012	Rapid effects of diverse toxic water pollutants on chlorophyll a fluorescence: variable responses among freshwater microalgae.	Water research (2012), Vol. 46, No. 8, pp. 2615-26	This article looks at effects of glyphosate + other compounds on the PSII system, determiningeffects to Chlorophyll A levels using fluorescence. Endpoints were generated using a novel approach that is not considered relevant to an EU level ecotoxicological risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Claassens A. et al.	2019	Soilborne glyphosate residue thresholds for wheat seedling metabolite profiles and fungal root endophyte colonisation are lower than for biomass production in a sandy soil.	Plant and Soil (2019), Vol. 438, No. 1/2, pp. 393	5.4.1 case b) Relevant but supplementary information: Presented information on effects of glyphosate on seedling emergence and soil fungi, but no specific endpoints are presented that could be used for the renewal ecotoxicological risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Claassens A. et al. 2019
Condrosari P. et al.	2018	Growth inhibition test of glyphosate herbicide for glyphosate-degrading- bacteria screening	International Journal of ChemTech Research (2018), Vol. 11, No. 5, pp. 240-248	The paper describes a screening test for establishing bacterial populations as tools for remediation of soils. The presented endpoints are not relatable to an EU level risk assessment from	The RMS does not agree with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				an ecotoxicological perspective.	This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Condrosari P. et al. 2018
Cordova Lopez A. M. et al.	2019	Exposure to Roundup® affects behaviour, head regeneration and reproduction of the freshwater planarian Girardia tigrina	Science of the total environment (2019), Vol. 675, pp. 453	5.4.1 case b) Relevant but supplementary information: This is an invasive flatworm species in the EU. No specific test guidelines are available for this type of study, despite the range of endpoints that appear to have been covered.	POEA is not authorized in plant protection products containing glyphosate (European Commission, August 2016). Due to presence of POEA, the study is considered not relevant by RMS. The reliability of the study was not assessed.
Cuhra M. et al.	2013	Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna.	Ecotoxicology (2013), Vol. 22, No. 2, pp. 251-62	Study was performed according to methods adapted from the ISO, US EPA and the OECD Testing. Juveniles > 24 hour old are not the approach advised in any of the test guidelines, so the acute results for the aged cohort studies cannot be related to an EU level risk assessment. Concerning the chronic exposure assay, this approach was modified from the guidelines stated above, extending beyond the 21 day duration of the guideline test. Validity criteria for the acute and chronic test were not stated. Details of the methods used to prepare the test media are not reported. Biological data are not reported for all age groups, so the data presented in the plots cannot be confirmed. The test organisms used in the tests were from different natural sources and poorly characterised as it would be needed to draw a regulatory relevant conclusion from the reported results. Furthermore and more critically, analytical dose confirmation of media in the vessels was not performed, so exposure cannot be confirmed. Due to the test materials not being the representative	The endpoint and chronic endpoints obtained from this study are considerably below those measured in regulatory studies. The use of older daphnids is not considered as a criteria of rejection by RMS. Despite the drawbacks listed by the applicant, RMS is of the opinion the study may still provide data to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Cuhra M. et al. 2013

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	
Currie Z. et al.	2015	Toxicity of Cuspide 480SL® spray mixture formulation of glyphosate to aquatic organisms.	Environmental toxicology and chemistry (2015), Vol. 34, No. 5, pp. 1178-84	The main focus of the article was a hazard assessment of two glyphosate formulations not available in Europe and the associated co-formulants. The study, measured toxicity values and calculated exposure values for South America. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Currie Z. et al. 2015
Dabney B. L. et al.	2018	Low-dose stimulation of growth of the harmful alga, Prymnesium parvum, by glyphosate and glyphosate-based herbicides.	Harmful algae (2018), Vol. 80, pp. 130	5.4.1 case b) Relevant but supplementary information: This paper does not present endpoints that can be used in the ecotox risk assessment for the renewal. The information are however considered supportive to discussions over hormesis.	It is hypothesized that glyphosate can become available to glyphosate-resistant phytoplankton and contribute to algal bloom development. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Dabney B. L. et al. 2018
da Costa Chaulet F. et al.	2019	Glyphosate- and Fipronil-Based Agrochemicals and Their Mixtures Change Zebrafish Behavior.	Archives of environmental contamination and toxicology (2019), Vol. 77, No. 3, pp. 443-451	This paper describes behavioural differences in zebra fish when exposed to either glyphosate or fipronil. No endpoint data presented could be used in an EU level for Annex I ecotoxicological risk assessment. Aversion / avoidance testing is not an EU level ecotoxicology risk assessment data requirement.	Zebrafish behavior was affected at 3 and 5 mg/L (of glyphosate based herbicide). They spent more time in the top zone and less time in the bottom zone. It is hypothesized by the study authors that in an environmental context, it can directly impair the ability of fish to avoid or evade predators. While RMS agrees with this, such effect cannot be directly linked to the population survival. No significant effect was noted at the concentration of 1 mg/L which is still above the concentration expected in the conditions of use. Overall, the

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					study does not provide relevant data to be used in the risk assessment.
da Cruz C. et al.	2016	Sensitivity, ecotoxicity and histopathological effects on neotropical fish exposed to glyphosate alone and associated to surfactant	Journal of Environmental Chemistry and Ecotoxicology (2016), Vol. 8, No. 3, pp. 25	5.4.1 case b) Relevant but supplementary information: The study was not conducted to GLP and/or according to a recognized test guideline and there are no validity criteria presented. The authors state that glyphosate alone and in association with Aterbane® BR was classified as practically non-toxic, whereas Aterbane® BR alone was considered moderately toxic for the tested organisms. However, due to insufficient explanation of experimental set-up (e.g. test substance, test medium, statistical analysis) and lack of experimental standard procedures (e.g. analytical verification), the study is may be used only as supportive information.	Only very high concentrations were tested i.e. 900 mg/L and above (on 3 fish species). LC50 was >975.0 mg L^{-1} of glyphosate (in formulation) for all species. Histopathological effects were noted, however the concentrations were far higher those expected in realistic conditions of use. RMS agrees that critical data are lacking. Overall the study does not provide relevant data for the risk assessment and is considered not reliable.
Damgaard C. et al.	2014	The effect of glyphosate on the growth and competitive effect of perennial grass species in semi-natural grasslands.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2014), Vol. 49, No. 12, pp. 897	5.4.1 case b) Relevant but supplementary information: Not directly relevant to Ecotox risk assessment, but maybe used in biodiversity discussion.	As highlighted by the applicant this study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Damgaard C. et al. 2014
da Rosa J. G. S. et al.	2016	Fish Aversion and Attraction to Selected Agrichemicals.	Archives of environmental contamination and toxicology (2016), Vol. 71, No. 3, pp. 415-22	Paper describes a novel fish avoidance study which is not considered relevant to an EU level risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
da Silva G. S. et al.	2019	Gene expression, genotoxicity, and physiological responses in an Amazonian fish, Colossoma macropomum (CUVIER 1818), exposed to Roundup and subsequent acute hypoxia	Comparative Biochemistry and Physiology, Part C: Toxicology & Pharmacology (2019), Vol. 222, pp. 49-58	The formulation used is based on MON 2139, which contains POEA. POEA surfactants are not present in the representative formulation (MON 52276) being used for the Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
da Silva R. A. et al.	2013	Compatibility of conventional agrochemicals used in rice crops with the entomopathogenic fungus *Metarhizium anisopliae*.	Scientia Agricola (2013), Vol. 70, No. 3, pp. 152-160	This paper presents the results of an agrochemical mixture study to entompathogenic fungus. As the study was performed using a mixture the appropriate endpoints for glyphosate cannot be determined.	The RMS agrees with the applicant's reasoning and conclusion.
Dalton R. L. et al.	2010	Comparison of the effects of glyphosate and atrazine herbicides on nontarget plants grown singly and in microcosms.	Environmental toxicology and chemistry (2010), Vol. 29, No. 10, pp. 2304-15	A study to look at the effects of Glyphosate (Roundup original + surfactant, 356 g/L) on single potted plant species compared with a microcosm. Based on relevant guidelines, six doses of up to 2136 g ai/ha label rate. IC25 results generated were used to compare test systems, however it is not possible to extrapolate to the risk assessments in the glyphosate renewal. Additionally, due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roundup Original, the study is considered not relevant by RMS.
de Brito Rodrigues L. et al.	2017	Ecotoxicological assessment of glyphosate-based herbicides: Effects on different organisms.	Environmental toxicology and chemistry (2017), Vol. 36, No. 7, pp. 1755-1763	The aim of the work presented in this paper was to evaluate the toxicity and potential effects of two glyphosate formulations on seed germination, brine shrimp and zebra fish larvae. The selected test species and design are not relatable to an EU level ecotoxicological risk assessment, as a USEPA approach was followed for a mixed consideration of diverse test species. The report provides insufficient description of study design and no specific rationale was cited for the formulations selected. Some methodology was performed according to OECD guidelines, however validity criteria were not evaluated and no analytical verification was performed.	Roundup Original, as stated in the report, contains POEA and is not considered relevant. The other formulation Glyphosate AKB 480 contains surfactants of unknown composition. This formulation is then considered "less relevant but supplementary" by RMS. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of de Brito Rodrigues L. et al.2017
de Campos Oliveira R. et al.	2016	Assessment of the potential toxicity of glyphosate-based herbicides on the	Phycologia (2016), Vol. 55, no. 5, pp. 577	5.4.1 case b) Relevant but supplementary information: Despite the study using a recognised OECD guideline, the endpoints in terms of	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		photosynthesis of Nitella microcarpa var. wrightii (Charophyceae)		respiration rates are not relevant to an EU level risk assessment for Annex I renewal, which specifically considers inhibition of glyphosate growth rates. The study considers technical glyphosate, Roundup and AMPA. Despite the techical material being identified, the formulation was not. It is not possible to conclude on the effects caused by the formulation as it was inferred that the product contains POEA.	
Deepananda K. H. M. A. et al.	2011	Acute toxicity of a glyphosate herbicide, Roundup (R), to two freshwater crustaceans.	Journal of the National Science Foundation of Sri Lanka (2011), Vol. 39, No. 2, pp. 169	.4.1 case b) Relevant but supplementary information: After exposure to Roundup® the 48 hour acute LC50 for adult copepod Phyllodiaptomus annae was determined to be 1.059 mg/L. This endpoint is questionable as there was only 19% mortality at the highest exposure concentration in the test (1.6 mg/L). For the second species, the 72 and 96 hour LC50 for decapod shrimp Caridina nilotica was determined to be 107.53 and 60.97 mg/L, respectively. However, the mean percenatge mortality at both timepoints was identical from Table 1 in the paper. As there are no biological data presented in the paper, the observed mortality and the LC50 calculation cannot be confirmed. The formulation content is identified as Roundup® (360g/L, 98%). However, the presented purity appears to be incorrectly stated, as a formulation with 98% purity, would suggest a technical material has been used, so there is uncertainty in actually what has been tested in the study. The tests were conducted according to EPA Guideline “Methods of Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms”. However, the origin of the organisms is not given. Therefore, previous exposure the test species may have had to pesticides or other chemicals is unclear. Furthermore, there was no	Due to potential presence of POEA and the doubtful results, the study is considered less relevant but supplementary (different formulation tested) and not reliable by RMS.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				analytical verification of test concentrations reported and the study is non-GLP, thus the reliability of the endpoint is questionable. Given the uncertainty in what was actually tested, the calculated endpoints and the conduct of the test, the study is considered unreliable.	
Demetrio P. M. et al.	2014	The effect of cypermethrin, chlorpyrifos, and glyphosate active ingredients and formulations on Daphnia magna (Straus).	Bulletin of environmental contamination and toxicology (2014), Vol. 93, No. 3, pp. 268	5.4.1 case b) Relevant but supplementary information: The test was not performed according to a relevant guideline. Although procedures are well documented, the water qualities during testing are not reported (only stock culture holding conditions are reported) and the test design in the study is not described, such as the number of animals exposed, test media preparation details and acclimation period prior to exposure. There are no biological data presented in order to confirm the achieved endpoints. The glyphosate formulation used in the testing is not the representative formulation for the renewal. Apparent from the endpoints achieved for the technical material and for the formulation, is the increased sensitivity of daphnia to the formulation, which is considered attributable to the co-formulants in the formulation and not to glyphosate. Based on the uncertainty associated with the materials and methods as described above, the study is considered as supplementary only.	Glyphosate (technical material) was used and the study may be considered " less relevant but supplementary". Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Demetrio P. M. et al. 2014
de Jesus Veloso Castro A. et al.	2015	Using a toxicity test with Ruppia maritima (Linnaeus) to assess the effects of Roundup.	Marine pollution bulletin (2015), Vol. 91, No. 2, pp. 506	5.4.1 case b) Relevant but supplementary information: This paper presents information on the effects of glyphosate on a saline tolerant species. However, there is no glyphosate exposure presented in the paper so it is very difficult to relate the observed effects to an exposure event / agricultural application	The aim of this study was to verify the acute effects of the formulated herbicide Roundup on the non-target species R. maritima under laboratory conditions (7 days exposure). No endpoint can be derived from the results as presented. No analytical verification was presented.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The study is considered not reliable by RMS
de Moraes C. P. et al.	2019	Hormetic effect of glyphosate on Urochloa decumbens plants.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2019), Vol. 55, No. 4, pp. 376-381	This paper presents a summary and review of hormetic growth response papers. No supportive data was presented to support stated endpoints.	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS.
Dennis P. G. et al.	2018	The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity	Scientific Reports (2018), Vol. 8, pp. 1	5.4.1 case b) Relevant but supplementary information: Nematode abundance is not an endpoint used in Ecotox risk assessment. However, these data are considered relevant to soil community effects based on single applications. Article is considered supplementary, as the approach used is not a recognised approach for ecotox risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Dennis P. G. et al. 2018
de Saraiva A. S. et al.	2016	Glyphosate sub-lethal toxicity to non- target organisms occurring in Jatropha curcas plantations in Brazil.	Experimental & applied acarology (2016), Vol. 70, No. 2, pp. 179-87	Endpoints not relatable to an EU level ecotoxicological risk assessment.	Roundup Transorb was used. Surfactants were not stated in this study. It is very likely that the tested formulation included the surfactant polyethoxylated tallow amine (POEA) as in an other study Rocha T. L. et al., 2015, (also conducted in Brazil) "Transorb" was used. In this study it was clearly stated that Transorb contains POEA. The study is considered not relevant by RMS.

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de Sousa Saraiva A. et al.	2015	Weed management practices affect the diversity and relative abundance of physic nut mites.	Experimental & applied acarology (2015), Vol. 65, No. 3, pp. 359-75	The paper describes a long term monitoring programme looking at weed management practices and their impact on mite species in a particular region of Brazil, that cannot be related to an EU level risk assessment.	Roundup Transorb was used. Surfactants were not stated in this study. It is very likely that the tested formulation included the surfactant polyethoxylated tallow amine (POEA) as in an other study Rocha T. L. et al., 2015, (also conducted in Brazil) "Transorb" was used. In this study it was clearly stated that Transorb contains POEA. The study is considered not relevant by RMS.
De Souza Filho J. et al.	2013	Mutagenicity and genotoxicity in gill erythrocyte cells of Poecilia reticulata exposed to a glyphosate formulation.	Bulletin of environmental contamination and toxicology (2013), Vol. 91, No. 5, pp. 583-7	Methods and endpoints are not relevant to an EU level ecotoxicology assessment.	Roundup Transorb was used. In this study it was clearly stated that Transorb contains POEA. The study is considered not relevant by RMS.
De Stefano L. G. et al.	2018	Comparative impact of two glyphosate-based formulations in interaction with Limnoperna fortunei on freshwater phytoplankton	Ecological indicators (2018), Vol. 85, pp. 575-584	This paper looks at the interaction of herbicide formulations in conjunction with the presence of mussels on the development of periphyton and phytoplankton communities. As the effects cannot be related directly to the single active substance, this paper is not considered relevant for the EU level Ecotoxicological risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of De Stefano L. G. et al. 2018
Debski H. et al.	2018	Effects of glyphosate and fluazifop-P- butyl on flavonoids content and growth of common buckwheat (Fagopyrum esculentum Moench)	Fresenius Environmental Bulletin (2018), Vol. 27, No. 1, pp. 91-97	Cellular level parameters discussed in the paper, with endpoints that are not relevant to an Annex I renewal from an ecotoxicological perspective.	The RMS agrees with the applicant's reasoning and conclusion.
Debski H. et al.	2018	Comparison of the response of seedlings of common buckwheat (*Fagopyrum esculentum* Moench) to glyphosate applied to the shoot or to the root zone.	Acta Agrobotanica (2018), Vol. 71, No. 1, pp. Article No.: 1730	Unable to establish the exposure rates used in the three different tests. mMolar solutions were prepared, but no attempt has been made to confirm dosing and no analysis performed. Endpoints are therefore not relevant to an EU level risk assessment from an ecotoxicological perspective.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Di Fiori E. et al.	2012	Impact of the invasive mussel *Limnoperna fortunei* on glyphosate concentration in water.	Ecotoxicology and environmental safety (2012), Vol. 81, pp. 106-13	Paper describes the use of golden mussels for removal of glyphosate from the water column. Endpoints presented cannot be be used in EU level Annex I renewal risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
do Carmo E. L. et al.	2010	Selectivity of pesticides used in soybean crops to trichogramma pretiosum riley, 1879 (hymenoptera: trichogrammatidae) pupae. Original title: seletividade de produtos fitossanitarios utilizados na cultura da soja para pupas de trichogramma pretiosum Riley	Arquivos do Instituto Biologico Sao Paulo (2010), Vol. 77, No. 2, pp. 282-290	Effects on parasitoid wasps via exposure of parasitised eggs which were immersed for 5 sec in test solutions. However, this is no adequate route of exposure and the content of active ingredient per area is unclear. Therefore the biological results cannot be attributed to a specific test concentration.	The RMS agrees with the applicant's reasoning and conclusion.
Dominguez A. et al.	2016	Toxicity of AMPA to the earthworm Eisenia andrei Bouche, 1972 in tropical artificial soil.	Scientific reports (2016), Vol. 6, pp. 19731	5.4.1 case b) Relevant but supplementary information: The study is well-documented and performed according to ISO guideline 11268-1 and 11268-2. However, the artificial soil used is not classed as representative in the EU. Soil characteristics are only partly given as information on CEC, organic carbon content and bulk density are missing. Additionally, one of the validity criteria for the chronic test was not met (the reported minimum number of control juveniles is too low). Endpoints (NOEC, LC50) were not derived and therefore this study delivers only supplementary information.	The RMS agrees with the applicant's reasoning and conclusion.
Dos Santos A. P. R. et al.	2017	A glyphosate-based herbicide induces histomorphological and protein expression changes in the liver of the female guppy Poecilia reticulata.	Chemosphere (2017), Vol. 168, pp. 933-943	The paper attempts to establish a proteomic method for detecting sub-lethal impacts of chemicals on fish. This is not relevant for risk assessment in the EU, where growth and reproductive parameters achieved in higher tier fish testing are considered. The formulation used is also not the representative formulation for the annex I renewal.	Roundup Transorb was used. In this study it was clearly stated that Transorb contains POEA. The study is considered not relevant by RMS.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Dos Santos Teixeira J. M. et al.	2018	Acute toxicity and effects of Roundup Original® on pintado da Amazonia.	Environmental science and pollution research international (2018), Vol. 25, No. 25, pp. 25383-25389	Endpoints presented were for a formulation that is not the representative formulation for the Annex I renewal.	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that states « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roundup Original, the study is considered not relevant by RMS.
Druart C. et al.	2012	Landsnail eggs bioassays: A new tool to assess embryotoxicity of contaminants in the solid, liquid or gaseous phase of soil	Applied soil ecology (2012), Vol. 53, pp. 56-64	Endpoints are not applicable to EU level ecotoxicology risk assessment. Approach described is novel and not validated.	The RMS agrees with the applicant's reasoning and conclusion.
Druart C. et al.	2017	A full life-cycle bioassay with Cantareus aspersus shows reproductive effects of a glyphosate-based herbicide suggesting potential endocrine disruption.	Environmental pollution (2017), Vol. 226, pp. 240	5.4.1 case b) Relevant but supplementary information: The test design is novel and the achieved endpoints cannot be used in an EU ecotoxicological regulatory risk assessment / glyphosate EU renewal.	Due to presence of POEA, the study is considered not relevant by RMS.
Druart C. et al.	2010	Towards the development of an embryotoxicity bioassay with terrestrial snails: screening approach for cadmium and pesticides.	Journal of hazardous materials (2010), Vol. 184, No. 1-3, pp. 26	5.4.1 case b) Relevant but supplementary information: Glyphosate active substance and glyphosate-based herbicide formulation Roundup® were tested to compare toxicity to land snails. LC50 valued were generated, however based on a new methodology (not to any established guideline). The metholodogy and endpoints generated are not relateable to an EU level ecotoxicological regulatory risk assessment for glyphosate EU renewal.	The aim of this study is to assess the embryotoxicity of chemicals with snail eggs (Helix aspersa aspersa Müller (syn. Cantareus aspersus asperses Müller, 1774 or Cornu aspersum). The effects of Roundup® Biovert 360 (360 g/l glyphosate; Monsanto Europe S.A.) and glyphosate were studied. Endpoints were the hatching success

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					and observations of embryo abnormalities after exposure. RMS noted that Roundup® Biovert 360 contains 360 g glyphosate / L as MON 52276. Roundup® Biovert 360 was found to be more toxic than glyphosate alone (EC50a.i. = 18 mg/l and about 1300 mg/l, respectively). EC10 of glyphosate to Helix aspersa was approximately 854 mg/L glyphosate (685–1348). RMS questions the relevance of the exposure. Four layers of paper (Quantitative filter paper grade 40 ashless, Whatman) dampened with 0.8 ml of control or contaminated solutions were laid on the bottom of the Petri dishes. Eggs were then placed in these Petri dishes until hatching. However snails lay their eggs in the topsoil (2–5cm depth). Thus the study design may influence what will happen in real conditions. It is agreed that they can be exposed to contaminants deposited on the ground and then leached downwards but the relevance of the aqueous solutions used in this study is questionable and not relatable to EU regulatory risk assessment. This study nevertheless highlights the need to assess the risk of the final product (which will be applied to crops) and not only of the active ingredient individually.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The study seems well conducted (despite the absence of specific guideline). However there are no details of biological observation reported in the paper. Thus, the LC50 calculation cannot be confirmed by RMS and the study design (paper in bottom of petri dishes) is questionable.. This study cannot be taken into account for the assessment of the active substance glyphosate itself. This study is considered not relevant by RMS.
Druart C. et al.	2011	Glyphosate and glufosinate-based herbicides: fate in soil, transfer to, and effects on land snails	Journal of soils and sediments (2011), Vol. 11, No. 8, pp. 1373	5.4.1 case b) Relevant but supplementary information: The material and methods part lack some important information. The test design for the exposure of snails to treated food is not specified and thus the intake dose per snail is unclear. Furthermore, the application of the test solutions into the soil is not reported and an even distribution cannot be confirmed. Nevertheless a chemical analysis of the soil during exposure was performed. As the biological data does not report results as an endpoint useful for the risk assessment, the study is not done to a guideline and is non-GLP and can be considered as supplementary only.	The presence of POEA is not mentioned in the study. However in an other study (Druart et al 2017) it was stated that the Roundup Bypass formulation contains POEA. The study is considered not relevant by RMS.
Druille M. et al.	2015	Glyphosate vulnerability explains changes in root-symbionts propagules viability in pampean grasslands	Agriculture, ecosystems & environment (2015), Vol. 202, pp. 48-55	Findings cannot be related to an EU level ecotoxicological risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Druille M. et al. 2015

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Druille M. et al.	2013	Arbuscular mycorrhizal fungi are directly and indirectly affected by glyphosate application	Applied soil ecology (2013), Vol. 72, pp. 143-149	Describes an experiment to establish if fungal hyphae associated with plant roots are affected by glyphosate. Endpoints achieved not relatable to EU level risk assessment. Exposure rates cannot be determined from the paper.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Druille M. et al. 2013
Du X. et al.	2012	Effects of eight herbicides on seed germination and seedling growth of Scutellaria baicalensis Georg	Xibei Nongye Xuebao (2012), Vol. 21, No. 4, pp. 202-206	The formulation (Glyphosate (Baron®) 48% SL (Elhelb), is not the representative formulation for the EU Annex I renewal. The study was not conducted to GLP and/or according to a recognized test guideline and there are no validity criteria presented. The authors state that Roundup had no effect on the germination of Scutellaria baicalensis Georgi seeds in laboratory petri dish test but inhibited the growth of Scutellaria baicalensis Georgi seedlings. However, given the lack of standard guidelines and important material and application methods, in conjunction with insufficiently reported test conditions and biological data, no useful endpoint for the risk assessment can be derived.	The RMS agrees with the applicant's reasoning and conclusion.
Dumitru G. et al.	2019	Effect of glyphosate herbicide on some hematological and biochemical parameters in Carassius auratus L	Revista de Chimie (2019), Vol. 70, No. 2, pp. 518-521	Sub-lethal effects on blood chemistry parameters are not relevant to an ecotoxicological risk assessment for the EU level renewal of glyphosate. On review of the report, the formulation was also a 48% a.e. content, with reasons for the observed effects related to POEA in the formulation described in the results. The representative formulation does not contain POEA, therefore results not relevant for the EU.	The presence of POEA was not reported in the study. The study is considered "less relevant but supplementary" by RMS. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Dumitru G. et al. 2019
Edge C. *et al.*	2014	Variation in amphibian response to two formulations of glyphosate-based herbicides.	Environmental toxicology and chemistry (2014), Vol. 33, No. 11, pp. 2628-32	Roundup WeatherMax and Roundup Weed and Grass Control, which are non EU representative glyphosate formulated products were used as the test substances. Prior exposure history to other	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable. The

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				chemicals and other organisms within their natural environment was unknown. Several limitations were observed within the study including lack of exposure history of the local organisms, inability to attribute the results entirely to the test substance, inability to develop a dose-response relationship or derive end-points within the study, the analytical approach and verification was lacking, and the study was not conducted according to a standard guideline.	study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
El Sebai O. A. et al.	2012	Side-effect of certain herbicides on egg parasitoid Trichogramma evanescens (West.) (Hymenoptera: Trichogrammatidae)	Academic Journal of Entomology (2012), Vol. 5, No. 1, pp. 1-10	The aim of the study was to compare the toxicity of four different commercially available herbicidal products to T. evanescens. Glyphosate was classified as harmless to T. evanescens wasps. The study was not conducted to a guideline or to GLP and the study design lacks some details compared with relevant guidelines. The test concentrations are based on nominal and no analytical verifications of test item concentrations were conducted. Only some details of the statistical analysis are reported. As the study is based on a glyphosate product, the toxicity of glyphosate active substance alone is unknown and therefore endpoints generated from this study are not quantifiable. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of El Sebai O. A. et al. 2012
Emmanuel L. D. A. et al.	2015	Effect of glyphosate on Bacillus megaterium with reference to tea ecosystem.	International Journal of Tea Science (2015), Vol. 11, No. 3/4, pp. 16	5.4.1 case b) Relevant but supplementary information: Endpoints are not releateable to an EU ecotox risk assessment, but may inform on discussions over community level effects in soil.	As highlighted by the applicant this study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Emmanuel L. D.A. et al. 2015

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Enemaduku A. M. et al.	2015	Effects of pesticides on the micro- flora of loamy soil obtained from Biological Garden, Federal University of Technology, Minna, Nigeria	Journal of Scientific and Engineering Research (2015), Vol. 2, No. 4, pp. 55-63	This monitoring study based on a Nigerian soil type, uses endpoints that are not applicable to an EU level ecotoxicological risk assessment.	This study is not directly relevant for the risk assessment but may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Enemaduku A. M. et al. 2015.
Erban T. et al.	2017	Detection of the desiccant and plant growth regulator chlormequat in honeybees and comb pollen.	Veterinarni Medicina (2017), Vol. 62, No. 11, pp. 596-603	Investigation of samples from hives exhibiting poisoning. Analyzed many pesticides (including glyphosate). No glyphosate detections reported.	The RMS agrees with the applicant's reasoning and conclusion.
Faghani M.	2018	Effect of glyphosate on honey bee (*Apis Mellifera*) performance	Arthropods (2018), Vol. 7, No. 3, pp. 77-81	Presents no data that can be used in an EU based risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Fagundez G. A. et al.	2016	Do agrochemicals used during soybean flowering affect the visits of Apis mellifera L.?	Spanish Journal of Agricultural Research (2016), Vol. 14, No. 1, p. e0301	5.4.1 case b) Relevant but supplementary information: Field level investigation where soybean are sprayed with glyphosate and the behaviour of bees is assessed. Findings not directly relateable to EU level risk assessment, as OTT crop application not on GAP - the observed effects are potentially useful for the discussion on indirect effects .	As highlighted by the applicant this study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Fagundez G. A. et al. 2016
Fai P. B. A. et al.	2015	Potential of the microbial assay for risk assessment (MARA) for assessing ecotoxicological effects of herbicides to non-target organisms.	Ecotoxicology (2015), Vol. 24, No. 9, pp. 1915-22	Novel test design / approach - not relatable to an EU level ecotoxicological risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Faita M. R. et al.	2018	Changes in hypopharyngeal glands of nurse bees (Apis mellifera) induced by pollen-containing sublethal doses of the herbicide Roundup	Chemosphere (2018), Vol. 211, pp. 566-572	This test was conducted using Roundup Original which contains POEA and is not therefore relevant to the EU level risk assessment for ANNEX I renewal.	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain

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					alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS.
Falis M. et al.	2014	Effects of heavy metals and pesticides on survival of Artemia franciscana.	Acta Veterinaria Brno (2014), Vol. 83, No. 2, pp. 95-99	This paper presents data for a formulation that cannot be related to an EU level risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Falis M. et al. 2014
Fan J. et al.	2013	Hydroxyl radical generation and oxidative stress in Carassius auratus exposed to glyphosate and its formulation	Toxicological and environmental chemistry (2013), Vol. 95, No. 7, pp. 1183-1191	Contains POEA, therefore not relevant to EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Fan J. Y. et al.	2013	Herbicide Roundup® and its main constituents cause oxidative stress and inhibit acetylcholinesterase in liver of Carassius auratus	Journal of environmental scienc and health, Part B. Pesticides, food contaminants and agricultural wastes (2013), Vol. 48, No. 10, pp. 844-850	Contains POEA, therefore not relevant to EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Farina W. M. et al.	2019	Effects of the Herbicide Glyphosate on Honey Bee Sensory and Cognitive Abilities: Individual Impairments with Implications for the Hive.	Insects (2019), Vol. 10, No. 10, pp. E354	This is a review article. No data presented that is supported.	The RMS agrees with the applicant's reasoning and conclusion.
Fedorova N. V. et al.	2019	Influence of glyphosate on the morphogenesis and biochemical indicators of onions and wheat.	Zashchita i Karantin Rastenii (2019), No. 9, pp. 47-48	Article concerns the effect of herbicide use on the nutrient content of wheat and onions. The endpoints / observations are not relatable to an EU level ecotoxicological risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Felix F. J. et al.	2015	Impact of the herbicide glyphosate roundup (41%) on the haematology of the freshwater fish, Catla catla (Hamilton)	IOSR Journal of Environmental Science, Toxicology and Food Technology (2015), Vol. 9, No. 4-3, pp. 56-60	Contains POEA, therefore not relevant to EU renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The presence of POEA is not stated in the report. However the results on haematological parameters are not relatable to the risk assessment. The study is considered not relevant.
Felix F. J. et al.	2018	Efficacy of herbicide glyphosate Hijack on the blood parameters of the freshwater fish, *Catla catla* (HAM)	Asian Journal of Biology (2018), Vol. 7, No. 2, pp. 38848	Biological impacts on enzyme levels in blood are not used in an EU level ecotoxicological risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Felline S. et al.	2019	The response of the algae *Fucus virsoides* (Fucales, Ochrophyta) to Roundup® solution exposure: A metabolomics approach.	Environmental pollution (2019), Vol. 254, No. Pt A, pp. 112977	Novel approach utilising metabolomics. The latter is not used in EU level risk assessment for Annex I renewal and is thus not releatable to the risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Felline S. et al. 2019
Ferreira E. A. et al.	2015	Cassava physiological responses to the application of herbicides. Respostas fisiologicas da mandioca a aplicacao de herbicidas.	Semina: Ciencias Agrarias (2015), Vol. 36, No. 2, pp. 645-655	Endpoints not relatable to an EU level ecotoxicological risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Ferreira E. A. et al. 2015
Ferreira-Junior D. F. et al.	2017	Low Concentrations of Glyphosate- Based Herbicide Affects the Development of Chironomus xanthus	Water, air, and soil pollution (2017), Vol. 228, No. 10, 390 p	The purpose of the study was to test acute and chronic toxicity of Roundup® Original to a tropical fresh water midge. Roundup Original contains POEA surfactant which is not permitted for use in formulations in the EU. The representative formulation (MON 52276) does not contain POEA. The influence of the surfactant on the achieved results in this study cannot be	The RMS agrees with the applicant's reasoning and conclusion. The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that states « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				excluded. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS.
de Souza Filho J. et al.	2013	Toxicological effects of a glyphosate- based formulation on the liver of *Poecilia reticulata*	Current Topics In Toxicology (2013), Vol. 9, pp. 81-91	The study was performed to assess the acute mortality (based on OECD 203) and sub-lethal effects (including histopathology). The study lacks several experimental standard procedures (e.g. analytical verification, reporting of validity criteria). Furthermore the formulation (Roundup Transorb) is not the representative formulation for the EU Annex I renewal (MON 52276) that contains POEA, a co-formulant that is not permitted in formulations in the EU. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Filippov A. A. et al.	2019	Effect of Roundup Herbicide on the Temperature Characteristics of Maltase of the Intestinal Mucosa in Juvenile Fish	INLAND WATER BIOLOGY (2019), Vol. 12, No. 2, pp. 248-253	Enzymatic impacts resulting from exposure are not considered in the EU level ecotoxicological risk assessment for Annex I renewal. It is extremely difficult to relate the findings to an EU level exposure scenario.	The RMS agrees with the applicant's reasoning and conclusion.
Filizadeh Y. et al.	2011	Toxicity determination of three sturgeon species exposed to glyphosate.	Iranian Journal of Fisheries Sciences (2011), Vol. 10, No. 3, pp. 383	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)	The study investigates the acute toxicity of glyphosate (in roundup formulation) to three different sturgeon species (Huso huso, Acipenser stellatus, and A. persicus) under laboratory conditions.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					96–h LC50 for H. huso, A. stellatus and A. persicus were 26.4, 23.2 and 27.5 mg.l–1, respectively. These LC50 values are below the other acute endpoints issued from other studies submitted for the EU representative formulation MON 52276. The formulation used in this study (Roundup) is not the EU representative formulation MON52276. In the discussion, the authors indicate that "the lethal toxicity of commercial toxicant formulation such as Roundup® is more than the glyphosate technical grade substance. The surfactants such as polyethoxylated tallowamine (POEA) used in the Roundup formulation are the principal toxic compound of the glyphosate–based herbicide to aquatic organisms". It is assumed by RMS that the Roundup formulation used in the test very (and likely contains contained surfactants such as POEA, according to the study authors). The presence of POEA was also assumed in RAR 2015. The study is then considered less not relevant. Moreover there is no chemical analysis reported in the report to confirm the exposure. Only two values were reported in the discussion part (8.31 to 77.21 mg/L) that seem to indicate rather low recovery (as tested concentrations were between 10-100 mg/L nominal). The authors also noted that many injury appearances were observed in Sturgeon fries in

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					whole concentrations during the study (the reason of that is unknown but this statement questions the condition of the fish in the test). No biological observations is reported, only LC50 values are given. The reliability can not be assessed properly. The study is considered less relevant but supplementary (due to the different formulation tested) and not reliable by RMS.
Fiorino E. et al.	2018	Effects of glyphosate on early life stages: comparison between *Cyprinus carpio* and *Danio rerio.*	Environmental science and pollution research international (2018), Vol. 25, No. 9, pp. 8542-8549	This paper is a poster abstract with no associated paper. There is insufficient information presented in the poster abstract to establish relevance of the poster to the Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Frontera J. L. et al.	2014	Effects of glyphosate and polyoxyethylene amine on metabolic rate and energy reserves of Procambarus clarkii juveniles.	Open Environmental Sciences (2014), Vol. 8, pp. 49-53	Contains POEA, therefore not relevant to EU renewal.	The RMS does not agree with the applicant's reasoning and conclusion. Glyphosate was also tested not in combination with POEA. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Frontera J. L. et al. 2014
Fuentes L. *et al.*	2011	Comparative toxicity of two glyphosate formulations (original formulation of Roundup® and Roundup WeatherMAX®) to six North American larval anurans.	Environmental toxicology and chemistry (2011), Vol. 30, No. 12, pp. 2756-61	The original formulation of Roundup and Roundup WeatherMAX are not the representative formulation for the Annex I renewal. The original formulation of Roundup used, contains a POEA surfactant, which is not permitted for use in the EU. The test design is well described in the paper, but due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the conclusion regarding Roundup. However, the results on Roundup WeatherMAX are considered less relevant but supplementary and reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9
Gabriel U. U. et al.	2010	Toxicity of roundup (a glyphosate product) to fingerlings	Animal Research International	5.4.1 case a) relevant and provides data for the risk assessment: Summary is provided in MCA 8	The conclusion given by the applicant is partial. Not only lethal effect but also sublethal, i.e. opercular beat

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		of Clarias gariepinus.	(2010), Vol. 7, No. 2, pp. 1184		frequency, tail beat frequency, are measured in this study. The relevance of these sublethal effects for the risk assessment cannot be established by RMS as no quantitative link can be made between these parameters and the potential adverse effect at population level (this latter being the specific protection goal). So only results on mortality were considered in deep by RMS. Nevertheless, a link between these abnormal behaviors may exist and may be indicative of mortality and/or potential adverse effect at population level in natural conditions. So, the results for sublethal effects were also reported in the summary and may be considered in future, together with other data available for the active substance. The present study assessed the acute toxicity (lethal and sublethal) of the glyphosate based formulation Roundup. RMS cannot check (based on the available information) if the surfactant polyethoxylated tallow amine (POEA) was present or not in the formulation. Since August 2016, POEA is not authorized in plant protection products containing glyphosate (European Commission). Based on the country and the year the study was conducted, it is likely that the formulation used contained POEA. POEA is known to be highly toxic to aquatic organisms. It is then

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					not known if the high toxicity measured in this study is due to the formulation (and its co-formulants) or a species-specific sensitivity. The study was conducted with the African catfish Clarias gariepinus. RMS considers that the sensitivity of this species can be considered representative of European catfish species. The applicant notes that there is no analytical verification of test concentrations reported and thus the reliability of the endpoint is questionable. RMS agrees that the absence of analytical verification is a severe drawback of the study. Dose relationship was observed indicating that dosing was somehow adequate, nevertheless uncertainty remains on the actual concentrations. The applicant notes that mucus accumulation on the skin and gills and skin pigmentation were recorded in fish in the holding / stock vessels. To RMS understanding, "recorded" only means that it was part of the study design (not that it was observed in control). The study author noted that mucus accumulation was concentration-dependant and minimal in the control. The authors derived what they called "Safe concentration" by multiplying the lethal concentration by a factor

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					0.1. RMS does not consider these values relevant for risk assessment. The dissolved oxygen value reported in the study is of 0.01 ± 0.05 mg/l. RMS considers this as a typing error (control fish would have not survived). The 96 hour LC50 of Roundup on the fish was 15.88 mg/l (equivalent to approximately 5.7 mg glyphosate acid equivalent/L). RMS cannot discard higher sensitivity of this species (which can be considered representative of European catfish species). However, the similarity of the formulations (Roundup vs. MON 52276) is not established. RMS considers this study being less relevant but supplementary (formulation issue). The data are considered not reliable.
Gagneten A. M. et al.	2014	Efectos del herbicida ron-Do® sobre cerodaphnia reticulata (crustacea, cladocera) y degradabilidad del glifosato (n- fosfometilglicina) en condiciones Experimentales	Natura Neotropicalis (2014), Vol. 45, No. 1&2, pp. 71-85	Formulation is not the representative formulation for the Annex I EU renewal.	The RMS considered the study as less relevant. The tested concentrations are 5.33; 15.99 and 31.98 mg of acid equivalent per liter. Specimens of C. reticulata used were obtained from samples taken from two lenitic lagoons (indicated as uncontaminated with pesticides) and acclimatized for 4 generations. The effectiveness of the acclimatation is unknown. The survival (90%) and number of neonates in control (135) suggest that the test was adequately performed. At these concentrations no effect were

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					reported on survival but impact on fertility (number of neonates and reproduction rate) was recorded. However, as presented (no details of observations per replicates, no table of results to allow recalculation); the results did not allow to derive endpoints for risk assessment such as NOEC or EC10.. Moreover the tested concentrations are far above the expected exposure concentrtaions. In addition, effects of co-formulants may influence the results of this chronic toxicity test. Overall, RMS considered that the study did not provide information useful for risk assessment of glyphosate and its representative formulation.
Galin R. R. et al.	2019	Effect of Herbicide Glyphosate on Drosophila melanogaster Fertility and Lifespan.	Bulletin of experimental biology and medicine (2019), Vol. 167, No. 5, pp. 663-666	The formulation used (GLYPHOS) contains POEA which is not relevant to the EU level ecotoxicological risk assessment for Annex I renewal, as the representative formulation does not contain POEA, which is a known surfactant this is known to be more toxic than glyphosate.	The RMS agrees with the applicant's reasoning and conclusion that formulation containing POEA are not relevant. Data gap: Provide a justification that the formulation Glyphos used in the paper of Galin R. R. et al. 2019 contains POEA.
Garcia-Espineira M. et al.	2018	Toxicity of atrazine- and glyphosate- based formulations on Caenorhabditis elegans.	Ecotoxicology and environmental safety (2018), Vol. 156, pp. 216-222	The formulated product used in the test contains MON 2139 which contains POEA (MON0818). Therefore findings are not relevant to the EU level andrepresentative formulation for the Annex I renewal.	The article did not mention the fact that the formulation contains POEA. However other papers are available that used the Roundup formulation for which it is clearly stated that the formulation contains POEA. Therefore the RMS considered the study not relevant.
Garcia-Perez J. A. et al.	2016	Impact of litter contaminated with glyphosate-based	Applied soil ecology (2016), Vol. 104, pp. 31-41	Relates to a long term monitoring study on earthworms specific to South America.	Specific condition of coffee plantation in South America. Substrate tested may contains different type of

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		herbicide on the performance of Pontoscolex corethrurus, soil phosphatase activities and soil pH			chemicals. Thus the RMS agrees with the applicant's reasoning and conclusion.
Garcia-Torres T. et al.	2014	Exposure assessment to glyphosate of two species of annelids.	Bulletin of environmental contamination and toxicology (2014), Vol. 93, No. 2, pp. 209	5.4.1 case b) Relevant but supplementary information: Information may be used to support the lack of effects in earthworm studies.	The study provided information on adult mortality, biomass, fecundity and viability of cocoons with in *Eisenia fetida* and *Octolasion Tyrtaeum* exposed to glyphosate. The method used follow EPA standards and analytical measurments have been parformed. The study is thus relevant and should be assessed for its reliability. A data gap is set. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Garcia-Torres T. et al. 2014.
Garza-Leon C. V. et al.	2017	Toxicity evaluation of cypermethrin, glyphosate, and malathion, on two indigenous zooplanktonic species.	Environmental science and pollution research international (2017), Vol. 24, No. 22, pp. 18123-18134	The tested formulation is not the representative formulation for the Annex I renewal.	The authors indicated that the tested formulation Faena® was found to be more toxic than glyphosate in previous experiment. Thus impact of co-formulants could not be excluded. Therefore, the RMS agrees with the applicant's reasoning and conclusion.
Gaupp- Berghausen M. et al.	2015	Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations.	Scientific reports (2015), Vol. 5, pp. 12886	Paper discusses indirect impact of nutrient loads in soil after GBH application. Not relatable to EU ecotoxicology assessment.	In a greenhouse experiment, the impact of Roundup (different formulations of Roundup applied within a week) on two earthworm species with different feeding strategies. Weed communities (grass, leguminous herb and non-leguminous herb species) were established in pots. Vertically burrowing or horizontally burrowing earthworm species were added.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Mesocosms were treated with a "lower-than-recommended" dose of glyphosate-based herbicide. 3 treatments (no earthworms, Lt, Ac) and two herbicide treatments (− H, + H) The main objective was to investigate the surface casting activity of the worms. Results: Vertically burrowing earthworms (Lumbricus terrestris) almost ceased activity three weeks after herbicide application, while the activity of soil dwelling earthworms (Aporrectodea caliginosa) was not affected. RMS notes that the reduced surface casting activity after herbicide treatment might be that L. terrestris avoided plant residues contaminated with glyphosate on the surface. As a consequence these earthworms might have lived in deeper soil horizons and avoided surface foraging and casting (as hypothetized by the authors). So the relevance of this parameter for the risk assessment is questionable. No quantitative link can be made with the protection goals. RMS further notes that at the end of the experiment, 93.3 ± 6.6% and 86.7 ± 9.9% of introduced numbers of L. terrestris and 100.0 ± 0.1% and 100.0 ± 2.6% of introduced numbers of A. caliginosa in –H and + H treatments were retrieved,

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					respectively. So no significant impact on survival was noted. Reproduction of the soil dwellers was reduced by 56% within three months after herbicide application. According to the authors, reproduction success of both earthworm species substantially decreased after herbicide application. 25 cocoons from L. terrestris (18 cocoons in two –H, 7 cocoons in one + H mesocosm) and 292 cocoons from A. caliginosa (193 cocoons in six –H, 99 cocoons in six + H mesocosms). RMS notes that 12 replicates were conducted for each treatment, so it appears that a number of replicates did not provide cocoons even in the non-treated pots. RMS doubts the reliability of the reproduction outputs. Hatching rate, i.e., percentage of cocoons from which earthworms hatched, decreased from 43% to 17% for L. terrestris (no statistical test was performed because of two few replications among treatments) and from 71% to 32% for A. caliginosa ($P < 0.001$) when cocoons were collected in mesocosms without herbicide or with herbicide treatment, respectively. This seems to indicate an effect on hatching, significant for A. caliginosa. Herbicide application led to increased soil concentrations of nitrate by 1592% and phosphate by 127%, pointing to potential risks for nutrient

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					leaching into streams, lakes, or groundwater aquifers. No biological data was presented. Besides RMS notes that the actual exposure cannot be quantified, no concentration measurements available. The application rate is not clear and involves several products applied successively. Weeds were well developed at the time of spraying, so interception occurred but it is not known to what extent. These uncertainties make this study not reliable. The study is considered less relevant but supplementary (uncertainty regrading formulation) and not reliable.
Ge HuiLin et al.	2014	Predicting joint toxicity of organophosphorus and triazine pesticides on green algae using the generalized concentration addition model.	China Environmental Science (2014), Vol. 34, No. 9, pp. 2413-2419	Discusses use of novel test approaches, not currently relevant to EU level risk assessment in ecotoxicology.	IC50 for single compound glyphosate is reported. The fact that the paper is related to a novel test approaches is not sufficient to exclude the study. A detailed assessment of relevance and reliability is requested. Data gap: Provide a summary and an assessment of relevance and reliability for the paper of Ge HuiLin et al., 2014.
Georgieva E. et al.	2018	Glyphosate-based herbicide alters the histological structure of gills of two economically important cyprinid species (common carp, cyprinus carpio and bighead carp, *Aristichthys nobilis*).	Applied Ecology and Environmental Research (2018), Vol. 16, No. 3, pp. 2295-2305	Observations in the paper cannot be related to the ANNEX I level EU risk assessment for renewal. Sub-lethal effects at the histopathological level are not considered in the EU level ecotoxicological risk assessment.	This study investigates the histopathological effects of a glyphosate based herbicide on the gills of two Cyprinid fish (common carp and bighead carp). The concentrations tested in laboratory conditions were high (72 mg/L, 40 mg/L and 20 mg/L), 96 hours.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Pathological alterations in the fish gills were observed. Bighead carp was more sensitive compared to common carp. The test item is not well defined. No analytical verification was reported. Fish were rather big: 17.6 cm ± 2.9 and mean body mass 46.3 g ± 8.4 for common carp and mean length 18.65 cm ± 1.33 and mean body mass 53.02 g ±5.3 for bighead carp. The study may not cover sensitivity of younger individuals. The pH, temperature, dissolved oxygen, oxygen saturation and conductivity were measured but values were not reported. Histopathological effects are not directly relatable to the risk assessment. Semi-quantitative scoring was used to quantify the degree of change of gill surface (this parameter is used to assess the histological alteration of the gills). These degree of severity as they are described (no effect, mild, moderate, severe, very severe) cannot be used in the standard risk assessment. RMS considers this appropriate to compare the sensitivity of the 2 species (main aim of this study) but of limited relevance for the risk assessment itself. The concentrations tested were far above those expected in environmentally realistic conditions, so the study is not considered relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Geyer R. L. et al.	2016	Effects of Roundup formulations, nutrient addition, and Western mosquitofish (Gambusia affinis) on aquatic communities.	Environmental science and pollution research international (2016), Vol. 23, No. 12, pp. 11729-39	Formulations used do not match that of the representative formulation for the Annex I renewal. Effects from co-formulants cannot be excluded.	The study is considered not relevant by RMS (formulation likely include containing POEA according to the study authors).
Gherhardt T. et al.	2011	Avoidance behavior of Eisenia foetida to acetone, deltamethrin and glyphosate	Annals of West University of Timisoara, Series of Chemistry (2011), Vol. 20, No. 2, pp. 1-10	This study was a new design to look at the avoidance of earthworms to chemicals. The study was not conducted to a known guideline. For the glyphosate part of the study, all the worms died due to heat/dehydration and so the effects of glyphosate were not clearly determined and the endpoints are not relevant to the regulatory risk assessment of glyphosate.	The RMS agrees with the applicant's reasoning and conclusion.
Gholami-Seyedkolaei S. J. et al.	2013	Effect of a glyphosate-based herbicide in Cyprinus carpio: assessment of acetylcholinesterase activity, hematological responses and serum biochemical parameters.	Ecotoxicology and environmental safety (2013), Vol. 98, pp. 135-41	Paper describes haematological and enzymatic biomarkers that could be used to assess the impact on fish in the field. There are no data presented that could be used in EU level Annex I renewal Ecotoxicological risk assessment.	The authors indivcated that "the formulation of Roundups is composed of isopropylamine (IPA) salt of glyphosate 480g/L, water and poly-ethoxylated amine surfactant (POEA) (Jiraungkoorskuletal., 2002),".). Due to the presence of POEA, the RMS agrees to consider the study as not relevant.
Gholami-Seyedkolaei S. J. et al.	2013	Optimization of recovery patterns in common carp exposed to roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters.	Ecotoxicology and environmental safety (2013), Vol. 98, pp. 152-61	Molecular level results that are not relatable to an EU level ecotoxicology risk assessment.	The authors indicated that the formulation tested contained Polyethoxylene amine (POEA). Thus, the RMS agrees to consider the study as not relevant.
Giaquinto P. C. et al.	2017	Effects of Glyphosate-Based Herbicide Sub-Lethal Concentrations on Fish Feeding Behavior.	Bulletin of environmental contamination and toxicology (2017), Vol. 98, No. 4, pp. 460-464	Test design and endpoints are not used in EU level risk assessment for annex I renewal.	The authors discussed the presence of surfactant in Roundup that may influence the toxicity. Other publications performed with roundup indicated the presence of POEA. Thus the RMS agrees to consider the paper not relevant here.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Givaudan N. et al.	2014	Earthworm tolerance to residual agricultural pesticide contamination: field and experimental assessment of detoxification capabilities.	Environmental pollution (2014), Vol. 192, pp. 9-18	Study provides information at the cellular/molecular level and is not an ecotoxicological relevant study	The formulation RounUp Fash was used. The authors suggested in the discussion that the membrane derangement for A. caliginosa by glyphosate is even more by its adjuvants in the formulation RoundUp Flash. Overall the results are considered not applicable for use in ecotoxicology risk assessment.
Gomes M. P. et al.	2017	Effects of glyphosate acid and the glyphosate-commercial formulation (Roundup) on *Dimorphandra wilsonii* seed germination: Interference of seed respiratory metabolism.	Environmental pollution (2017), Vol. 220, No. Pt A, pp. 452-459	Findings are not relatable to an EU level Annex I risk assessment as this species is only found in Brazil.	The study authors investigated the effects on seed germination and seed respiratory of glyphosate and the formulation Roundup. The results may provided information on seed germination. Even if the species tested is a Brazilian one it may be used as indicator for other species. Thus further consideration are considered necessary. Data gap: provide a summary and an assessment of relevance and reliability of the paper of Gomes M. P. et al. 2017.
Griesinger L. M. et al.	2011	Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems.	Chemosphere (2011, Vol. 84, No. 10, pp. 1461- 6	Study looks at the potential impact of glyphosate product on wolf spider mate location. Conducted in the US. No relevant endpoints generated for use in the risk assessment for the renewal of glyphosate.	Due to the presence of POEA in the tested formulation, the study is considered not relevant.
Grzesiuk A. et al.	2018	Effect of root-zone glyphosate exposure on growth and anthocyanins content of radish seedlings	Acta scientiarum polonorum- hortorum cultus (2018), vol. 17, no. 2, Pp. 3-10	Unable to establish what exposure concentrations were used in the study. Therefore not relatable to an EU level risk assessment for EU renewal.	Radish cultivated under hydroponic conditions. Test design not relevant (paper filter). The RMS considered the study not reliable.
Guijarro K. H. et al.	2018	Soil microbial communities and glyphosate decay in soils with different herbicide application history.	The Science of the total environment (2018), Vol. 634, pp. 974-982	Soil dissipation in Argentina is difficult to relate and thus not relevant to EU risk assessment.	Use of agricultural Argentinian field soils that may contains mixture of compound as soils are from conventional agriculture.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The RMS considered the study not relevant for renewal of glyphosate approval.
Guilherme S. et al.	2014	DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)--the major environmental breakdown product of glyphosate.	Environmental science and pollution research international (2014), Vol. 21, No. 14, pp. 8730- 9	The study assessed the impact of AMPA on Anguila anguila using COMET and ENA assays. The assays are not considered relevant to the ecotoxicological risk assessment for Annex I renewal. Therefore this paper should be considered non-relevant.	The tested formulation Roundup Ultra is indicated to contain POEA. Thus the study is not relevant.
Gungordu A.	2013	Comparative toxicity of methidathion and glyphosate on early life stages of three amphibian species: *Pelophylax ridibundus*, *Pseudepidalea viridis*, and *Xenopus laevis*.	Aquatic toxicology (2013), Vol. 140-141, pp. 220-228	5.4.1 case b) Relevant but supplementary information. Acute endpoints for amphibians are not a data requirement for the EU ecotoxicological regulatory risk assessment, as there are no recognised guidelines. The glyphosate formulation used is not the representative formulation for the glyphosate EU renewal.	The results are not considered relevant for the assessment of the representative product since the tested formulation includes substances that are similar to substances that are not allowed in the EU (Regulation (EU) 2016/1313 and/or DRAFT Regulation amending Annex III of Regulation (EC) 1107/2009).
Gutierrez M. F. et al.	2017	Disruption of the hatching dynamics of zooplankton egg banks due to glyphosate application.	Chemosphere (2017), Vol. 171, pp. 644-653	Endpoints based on abundance are used in EU level ecotoxicological risk assessment. The formulation used is not the representative formulation and therefore the impact of co-formulants cannot be excluded. Therefore this study is not relevant to the Annex I renewal.	The study may be considered less relevant but supplementary in view of RMS criteria for relevance. Thus the relevance and reliability should be detailed. Data gap: Provide a summary and an assessment of relevance and reliability of the paper of Gutierrez M. F. et al 2017.
Hackenberger Davorka K. et al.	2018	Acute and subchronic effects of three herbicides on biomarkers and reproduction in earthworm Dendrobaena veneta.	Chemosphere (2018), Vol. 208, pp. 722	5.4.1 case b) Relevant but supplementary information: The chronic test was performed according to OECD 222. However, the study was not conducted to GLP. Information on validity criteria are missing, and there is not analytical verification of soil concentrations. The unexpectedly high number of cocoons and the low number of juveniles being produced in the control group at the end of the study suggests that the quality of the earthworms going into the	The RMS agrees that uncertainty exists on the reliability of the results due to the performance of the control. The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				study may have been low. According to OECD 222, by the end of the test, the number of juveniles produced per adult worm should be > 30. In this case, with six adult worms per replicate there was a mean production (juveniles per worm) of 2.67 worms per adult. It is also understood that the OECD 222 test guideline uses a different species (Eisenia fetida) and not Dendrobaena veneta. It is relevant to consider juvenile production in the control as a check on the test system robustness. This cannot be confirmed in this case. Therefore, the study can be considered acceptable as supplementary information.	
Hagner M. et al.	2019	Effects of a glyphosate-based herbicide on soil animal trophic groups and associated ecosystem functioning in a northern agricultural field	Scientific Reports (2019), Vol. 9, No. 1, pp. 1-13	This study looked at the effect of Roundup + hoeing on soil organisms. Effects on soil organisms based on Roundup alone cannot be determined from the presented data test groups. The test substance used is also based MON 78294 which is not the representative formulation for the Annex I renewal.	The tested formulation was Roundup Gold, which contains a surfactant, etheralkylamine ethoxylate (POEA) according to the authors. Thus the study is not relevant for renewal of glyphosate formulation in EU.
Hansen L. R. et al.	2016	Behavioral responses of juvenile *Daphnia magna* after exposure to glyphosate and glyphosate-copper complexes.	Aquatic toxicology (2016), Vol. 179, pp. 36	5.4.1 case b) Relevant but supplementary information: Paper considers the influence of metals in daphnia testing and their influence on toxicity. Soils on the toxicity of endpoints considering speciation and enhanced toxicity in the presence of metals are not used in the EU level ecotox risk assessment.	The study reported also results of glyphosate alone by analyzing behavior of Daphni magna related to its mobility. This could be of interest for investigation in weight of evidendce in support of the regulatory study. Further investigations is needed. Data gap : Provide a summary and assessment of relevance and reliability for the paper of Hansen L. R. et al. 2016.
Hasan F. et al.	2016	Ecotoxicological hazards of herbicides on biological attributes of *Zygogramma*	Chemosphere (2016), Vol. 154, pp. 398-407	Novel surface residue exposure study that presents endpoint data that is not relatable to the EU level risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This result is not directly relevant for the risk assessment but may provide

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		bicolorata Pallister (Coleoptera: Chrysomelidae).			relevant information to be used in a weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Hasan F. et al. 2016
Hefnawy M. A. et al.	2012	Interaction of some herbicides with phosphate solublization by Aspergillus niger and Aspergillus fumigatus.	Australian Journal of Basic and Applied Sciences (2012), Vol. 6, No. 10, pp. 518-524	Findings are not directly related to the effects of glyphosate on the organism.	The RMS agrees with the applicant's reasoning and conclusion.
Helander M. et al.	2019	Glyphosate residues in soil affect crop plant germination and growth.	Scientific reports (2019), Vol. 9, No. 1, pp. 19653	5.4.1 case b) Relevant but supplementary information: The study presents endpoints that may be considered relevant to a risk assessment, however, the test design does not reflect the seedling emergence study required as part of the data requirements.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information to be used in a weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Helander M. et al. 2019
Herbert L. T. et al.	2014	Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour.	The Journal of experimental biology (2014), Vol. 217, No. Pt 19, pp. 3457-64	Endpoints described are not currently relevant to EU level ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information to be used in a weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Herbert L. T. et al. 2014
Hill M. P. et al.	2012	Toxic effect of herbicides used for water hyacinth control on two insects released for its biological control in South Africa	Biocontrol science and technology (2012), pp. 1321-1333	Non-EU monitoring study. Extrapolation to EU is difficult.	The RMS agrees with the applicant's reasoning and conclusion.
Hong Y. et al.	2018	Assessment of the oxidative and genotoxic effects of the glyphosate- based herbicide roundup on the freshwater shrimp, Macrobrachium nipponensis.	Chemosphere (2018), Vol. 210, pp. 896-906	Study conducted using a formulation of glyphosate that is not the representative formulation for the EU renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Hong Y. et al. 2018
Houssou A. M. et al.	2017	Lethal and sub-lethal effects of cypermethrin and glyphosate on the freshwater's copepod, *Acanthocyclops robustus*.	ISJ-Invertebrate Survival Journal (2017), Vol. 14, pp. 140-148	The test species selected is also not described and environmental holding conditions (water quality) prior to and during the study were not indicated). The formulation (Kumark® (480 g/L) is not the representative formulation for the EU Annex I renewal (MON 52276). The study was not conducted to a guideline, but the acute toxicity test can be considered in-line with OECD guideline 202. According to OECD 202, the validity criteria are not met for Glyphosate (> 10 % mortality in the control). Additionally, there were no quantifiable endpoints presented in the paper to a non-standard species. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Houssou A. M. et al. 2017
Hued A. C. et al.	2012	Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of Jenynsia multidentata (Anablepidae, Cyprinodontiformes).	Archives of environmental contamination and toxicology (2012), Vol. 62, No. 1, pp. 107-17	Not the representative formulation. The formulation Roundup Max is based on MON 14420, which is not MON 52276, the representative formulation used in the renewal process.	The aim of this study was to evaluate the histological lesions of the neotropical native fish, Jenynsia multidentata, in response to acute and subchronic exposure to Roundup Max and to determine if subchronic exposure to the herbicide causes changes in male sexual activity of individuals exposed to a sublethal concentration (0.5 mg/l) for 7 and 28 days. It has been shown that the sexual activity of males was affected. RMS considers this is relevant parameter. Roundup Max granular (Monsanto, Argentina) was used, containing 74.7

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					% Glyphosate and 25.3 % surfactants (likely POEA). Therefore it is not possible to discriminate between effects due to glyphosate and the ones due to surfactants. The presence of POEA was also assumed in RAR 2015. The representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. There was no analytical verification and no measurement of water quality parameters. Overall the study is considered not reliable. The study is considered not relevant and not reliable by RMS..
Iannilli V. et al.	2019	Genotoxic effects induced by glyphosate-based herbicide on two gammarid species: the invasive Dikerogammarus villosus (Sowinsky, 1894) (Crustacea, Amphipoda) and the native Echinogammarus veneris (Heller, 1865).	Fundamental and Applied Limnology (2019), Vol. 193, No. 2, pp. 143-153	Endpoints cannot be used in an EU ecotoxicological risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Imre P. *et al.*	2018	Toxicity test of individual and combined toxic effects of herbicide Amega and copper-sulphate on pheasant embryos. Original title: Amega gyomirto szer es a rez-szulfat egyedi es egyuttes mereghatasanak vizsgalata facanembriokban.	Novenyvdelem (2018), Vol. 54, No. 11, pp. 476-482	The formulation tested (Amega) is not the representative formulation for the glyphosate EU renewal. Partly, the observations are caused by mixture of compounds / potentially causal factors and thus not attributable to a substance of concern (e.g. mixture toxicity).	The exposure pathway (immersion in glyphosate solution) is rather extreme and unlikely to occur in the field, and thus the RMS considers this study to be not relevant.
Iori S. et al.	2019	The effects of glyphosate and AMPA on the mediterranean mussel *Mytilus*	Environmental research (2019), Vol. 182, pp. 108984	Paper discusses the effects of glyphosate at the molecular level which not used in an EU level assessment or renewal.	The RMS does not agree with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		galloprovincialis and its microbiota.			This study may provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Iori S. et al. 2019
Isaac A. O. et al.	2017	Behavioural and some physiological assessment of glyphosate and paraquat toxicity to juveniles of African catfish, Clarias gariepinus.	Pakistan Journal of Zoology (2017), Vol. 49, No. 1, pp. 183	5.4.1 case b) Relevant but supplementary information: Although the study itself is not directly relatable to an EU level ecotoxicological risk assessment, the study was considered as supplementary only (EFSA GD Point 5.4.1 - relevance category B) as sub-lethal effects on fish behaviour following exposure to glyphosate were described.	The relevance of the effects on behaviour (hyperactivity, abnormal swimming, restlessness, loss of equilibrium) for the risk assessment cannot be established by RMS as no quantitative link can be made between these parameters and the potential adverse effect at population level (this latter being the specific protection goal). So only results on mortality were considered by RMS (even if other sublethal parameters are also likely to affect populations, they are not relatable to the risk assessment scheme). The present study shows that acute concentrations of glyphosate are toxic to African catfish Clarias gariepinus. After 96 h of exposure, the 96 h LC50 for glyphosate was found to be 0.530 mg/L. RMS notes that mortality data are identical to those presented in an other study conducted years earlier (Ayanda O. I. et al., 2015, Acute toxicity of glyphosate and paraquat to the African catfish (Clarias gariepinus, Teugels 1986) using some biochemical indicators). This study was also assessed by RMS and again, no test substance information was available.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Exposure is expressed as glyphosate but it cannot be ascertain that commercial formulation (containing surfactants) was not used. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. No analytical verification is available. RMS considers that the absence of analytical verification is a severe drawback of the study, particularly when considering the uncertainty regarding the test item used. Dose effect relationship was observed indicating that dosing was somehow adequate, nevertheless uncertainty remains on the actual concentrations.Therefore given the uncertainties on the test item together with the absence of analytics RMS considers that the reliability of the results is very limited. Thus, it cannot be taken into account as critical information or the assessment of the active substance glyphosate itself. This study is considered less relevant but supplementary (due to the uncertainty on the test item) and not reliable for risk assessment.
Issa A. A. E. et al.	2013	Alterations in some metabolic activities of Scenedesmus quadricauda and Merismopedia glauca in response to glyphosate herbicide.	Journal of Biology and Earth Sciences (2013), Vol. 3, No. 1, pp. B17	5.4.1 case b) Relevant but supplementary information: The reported endpoints in terms of growth rates and pigment levels are not relateable to the EU level risk assessment for the renewal. The identity of the test items cannot be confirmed.	This study investigated relevant parameters but the concentrations are not representative of those expected in realistic conditions of use: High 600, Medium 300 and Low 150 ppm for Scenedesmus and High 150, Medium 75 and Low 37.5 ppm for Merismopedia).

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					This study does not provide relevant data for the risk assessment.
Iummato M. M. et al.	2013	Evaluation of biochemical markers in the golden mussel Limnoperna fortunei exposed to glyphosate acid in outdoor microcosms.	Ecotoxicology and environmental safety (2013), Vol. 95, pp. 123-9	Cellular level endpoints cannot be related to the Ecotoxicology Annex I renewal risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Jacques M. T. et al.	2019	Reprotoxicity of glyphosate-based formulation in Caenorhabditis elegans is not due to the active ingredient only.	Environmental pollution (2019), Vol. 252, No. Pt B, pp. 1854	5.4.1 case b) Relevant but supplementary information: The toxicity of glyphosate (glyphosate in monoisopropylamine salt) and its commercial formulation Termifin - Dexter Latina to the nematode Caenorhabditis elegans was investigated. Reproductive capacity was evaluated by means of brood size. The material and methods section lack some important information. The preparation of the test solutions and application of the test item are not described. Test concentrations, controls and loading per replicate are not specified and therefore not verifiable. Description of exposure throughout the study is also missing. The formulation used is not the representative formulation for the renewal. Furthermore, no useful endpoint for the regulatory risk assessment of terrestrial organisms can be derived.	The RMS agrees with the applicant that no useful endpoint for the regulatory risk assessment of terrestrial organisms can be derived (organisms were exposed in water). The study is then not relevant for the risk assessment.
Jain S. et al.	2012	Herbicidal action on germination, amylase activity and gibberellin level in Cajanus cajan (L.).	Bioscience Discovery Journal (2012), Vol. 3, No. 2, pp. 232	5.4.1 case b) Relevant but supplementary information: The study was not conducted to GLP and the test substance source and identity could not be verified. The study has not been conducted according to a recognized test guideline and there are no validity criteria presented. The authors state that glyphosate affects the level of gibberellin and amylase activity, as well as causing the food reserve content of seedlings to decrease gradually with increase in concentration. However, given the lack of standard guidelines, unclear experimental design and approach, test substance and dose rates not sufficiently being reported as	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				well as challenges in interpreting the study results, make reaching any reliable conclusions from the study quite challenging.	
Janben R. et al.	2019	A Glyphosate Pulse to Brackish Long- Term Microcosms Has a Greater Impact on the Microbial Diversity and Abundance of Planktonic Than of Biofilm Assemblages	FRONTIERS IN MARINE SCIENCE (2019), Vol. 6, Article 758	Paper discusses a novel technique to monitor the effects of herbicide on brackish proteo bacteria and bacterial communities measuring 16S rRNA genes in samples of water accompanied by total cell counts and using operational taxonomic units. Whilst informative techniques were used, these data are not relevant to an EU level Annex I ecotoxicological risk assessment according to the 1107/2009 data requirements.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Janben R. et al. 2019
Janssens L. et al.	2017	Stronger effects of Roundup than its active ingredient glyphosate in damselfly larvae.	Aquatic toxicology (2017), Vol. 193, pp. 210-216	Formulation tested contains POEA which is not present in the representative product in the EU renewal.	The RMS agrees that the tested formulation contains POEA according to the authors. However results are alos presented for glyphosate alone. Thus further investigation is requested. Data gap : Provide a study summary together with assessment of relevance and reliability for the paper of Janssens L. et al. 2017.
Jaskulski D. et al.	2011	Effect of pre-harvest glyphosate application on grain germination and emergence of winter wheat self-sown plants. Wpyw glifosatu stosowanego przed zbiorem na kiekowanie ziarna i wschody samosiewow pszenicy ozimej.	Progress in Plant Protection (2011), Vol. 51, No. 2, pp. 927-931	Roundup energy (450 SL) is the test substance in this study which is not the representative product for the renewal of glyphosate. The study is conducted in winter wheat, this is not a use on the representative GAP table for the renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. The study may still provide relevant data for the risk assessment of non-target plants. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Jaskulski D. et al. 2011
Jarmul- Pietraszczyk J. et al.	2012	Herbicide toxicity to the California earthworms Eisenia fetida Sav. and Dendrobaena veneta Rosa	Ecological Chemistry and Engineering A (2012), Vol. 19, No. 9, pp. 1133	5.4.1 case b) Relevant but supplementary information: This study compared the toxicity of three different commercially available formulations on the reproduction of earthworms,	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				among them a glyphosate containing product (Glifocyd 360 SL). Further detail on active substance content, source and storage conditions were not provided. The study was not conducted according to a recognized test guideline nor under GLP. The origin of the earthworm species and their environmental holding conditions prior to and during the study have not been included. Information on the test soil characteristics is also missing and application of the test item to the soil is not described in detail. Sublethal and reproductive parameters of the control were reported, but information about control mortality is missing. In the chronic test only one single test item concentration was tested, with this information for the acute study missing. The endpoint generated from this study is given in mg/L and it is not clear how it can be transferred to soil concentrations as the bulk density in the test system is unknown and the statistical analysis is not provided in detail. Therefore, the endpoint presented is considered unreliable.	
Jayawardena U. A. et al.	2016	Combined Effects of Pesticides and Trematode Infections on Hourglass Tree Frog *Polypedates cruciger*	EcoHealth, (2016) Vol. 13, No. 1, pp. 111 22	The tested glyphosate formulation Roundup contains POEA as a surfactant system. Studies which can be difficult to extrapolate to EU (e.g. with local native species, geo climatic properties, land uses and agricultural practices, non EU monitoring data, residue definitions differing from EU).	The RMS agrees with the applicant's conclusion. The results are not considered relevant for the assessment of the representative product since the tested formulations include surfactants that are similar to substances that are not allowed in the EU (Regulation (EU) 2016/1313 and/or DRAFT Regulation amending Annex III of Regulation (EC) 1107/2009).
Jenkins M. B. et al.	2017	Impact of glyphosate-resistant corn, glyphosate applications and tillage on soil nutrient	Pest management science (2017), Vol. 73, No. 1, pp. 78-86	Long term monitoring study that is not relevant for ecotoxicological risk assessment for Annex I glyphosate renewal.	The RMS does not agree with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		ratios, exoenzyme activities and nutrient acquisition ratios.			This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Jenkins M. B. et al. 2017
Jesenska S. et al.	2011	Species Sensitivity Distribution (SSD) - application in environmental risk assessment of pesticides in European rivers. Distribuce citlivosti druhu (Species Sensitivity Distribution - SSD) - vyuziti pro hodnoceni rizik pesticidu v evropskych rekach.	Bulletin - VURH Vodnany (2011), Vol. 47, No. 3, pp. 29-38	Data for glyphosate was used in a SSD model to look at the river ecosystem (in Belgium). Concentrations were monitored at locations with the river basin and used in the model. Results were not relevant for the risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Jiang J. et al.	2017	Influence of commonly used pesticides on acute toxicity to earthworm Eisenia fetida and alteration of antioxidant enzyme activities.	Journal of Agro-Environment Science (2017), Vol. 36, No. 3, pp. 466-473	Acute toxicity to earthworms is not a data requirement in the EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the applicant's justification.
Jin J. et al.	2018	Sub-lethal effects of herbicides penoxsulam, imazamox, fluridone and glyphosate on Delta Smelt (Hypomesus transpacificus).	Aquatic toxicology (2018), Vol. 197, pp. 79-88	Presented endpoints based on cellular levels of enzymes cannot be related to an Ecotoxicological risk assessment for Annex I renewal.	The RMS agrees with the applicant's justification.
Jofre D. M. et al.	2013	Fish Toxicity of Commercial Herbicides Formulated With Glyphosate	Journal of Environmental & Analytical Toxicology. Vol. 4, no. 1, pp. 1	5.4.1 case b) Relevant but supplementary information: The test design and the achieved endpoints are not used in the EU ecotoxicological regulatory risk assessment.	The acute and chronic toxicity of two commercial formulations (Glacoxan® and Estrella®) was investigated. The acute toxicity was tested toward two fish species Danio rerio and Poecilia reticulata by evaluating the mortality. The chronic toxicity was assessed in D. rerio by measuring the biochemical parameters glutamic pyruvic transaminase (AST) and glutamic oxaloacetic transaminase (ASL).

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The relevance of the biochemical parameters for the risk assessment cannot be established by RMS as no quantitative link can be made between these parameters and the potential adverse effect at population level (this latter being the specific protection goal). So only results on mortality were considered by RMS. In the present study, for the two fish species, Glacoxan® produced 100% of mortality at 100 µL/L and 0% mortality at 50 µL/L (equivalent to 50 mg/L). No intermediate concentration was tested so no robust endpoint can be derived. Estrella® produced 100% of mortality at 50 µL/L and 0% mortality at 25 µL/L (equivalent to 25 mg/L) on Danio rerio. No intermediate concentration was tested so no robust endpoint can be derived. It produced 100% of mortality at 100 µL/L and 0% mortality at 50 µL/L (equivalent to 50 mg/L) on Poecilia reticulata. Again ,no intermediate concentration was tested so no robust endpoint can be derived. The nature of surfactant present in the formulations tested is unknown. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. This affects the relevance of the study.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					No analytical verification is available. No robust endpoints (LC50) could be derived from the study (as explained above). Thus, RMS considers that this study is not reliable and cannot be taken into account for the assessment of the active substance glyphosate itself. The study is considered less relevant (different formulation tested) but not reliable.
Jofre D. M. et al.	2015	Acute and chronic toxicity of glyphosate to native fish from San Luis province, Argentina	Current Topics In Toxicology (2015), Vol. 11, pp. 49-54	The tested formulation contains POEA and is therefore not relevant to the MON 52276 representative formulation for the Annex I renewal.	Due to the presence of POEA in the formulation indicated by the authors, the study is not relevant for EU renewal approval of glyphosate.
Jones D. K. *et al.*	2010	Roundup and amphibians: the importance of concentration, application time, and stratification.	Environmental toxicology and chemistry (2010), Vol. 29, No. 9, pp. 2016-25	Glyphosate product tests performed with larval amphibians (wood frog and American toads) in an outdoor mesocosms in the US. Up to 3 mg ae/L was applied at 0, 7 and 14 days to the mesocosm, and replicated. Egg masses were collected from nearby ponds and hatched in culture ponds with aged well-water. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Jones D. K. *et al.*	2011	Competitive stress can make the herbicide Roundup® more deadly to larval amphibians.	Environmental Toxicology and Chemistry (2011), Vol. 30, No. 2, pp. 446-454	This study assessed competition as a stressor in conjunction with Roundup treatment in an outdoor mesocosm (USA) containing different densities of tadpoles (green frogs, gray tree frogs, american bullfrogs). Glyphosate product was applied up to 3 mg ae/L for 7 dayswith replication. Egg masses were collected from nearby ponds and hatched in wading pools with aged well-water. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Karahan A. et al.	2018	Determination of the effect of some pesticides on honey bees.	International Journal of Agriculture, Environment and Food Sciences (2018), Vol. 2, No. 3, pp. 104-108	No effects observed from glyphosate exposure on body movement, however, endpoint not relevant for an EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Kelly D. W. et al.	2010	Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival	Journal of applied ecology (2010), Vol. 47, No. 2, pp. 498-504	Snails collected from a river in New Zealand. Study looks at exposure to glyphosate + POEA surfactant (diluted to 0.36 mg a.i./L), and parasite infection with particular emphasis on spinal malformation and survival of juvenile fish.The study also looks at the influence of glyphosate concentration on the rate of infection and survival of P.antipodarum snails. The paper does not contribute to the renewal of glyphosate in the EU.	Due to the presence of POEA in the formulation indicated by the authors, the study is not relevant for EU renewal approval of glyphosate.
Kennedy E. et al.	2012	Herbiciding *Phragmites australis:* effects on litter decomposition, microbial biomass, and macroinvertebrate communities.	Fundamental and Applied Limnology (2012), Vol. 180, No. 4, pp. 309	5.4.1 case b) Relevant but supplementary information: This paper provides information that is considered relevant to the biodiversity.	As highlighted by the applicant, this study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Kennedy E. et al. 2012
Khan A. et al.	2016	Comparative Study of Toxicological Impinge of Glyphosate And Atrazine (Herbicide) on Stress Biomarkers; Blood Biochemical and Haematological Parameters of the Freshwater Common Carp (Cyprinus carpio).	Polish Journal of Environmental Studies (2016), Vol. 25, No. 5, pp. 1995-2001	Molecular and chemical observations are not relevant to traditional ecotoxicological risk assessment. Population level effects may not be inferred from such observations.	The RMS agrees with the applicant's reasoning and conclusion.
Kielak E. et al.	2011	Phytotoxicity of Roundup Ultra 360 SL in aquatic ecosystems: Biochemical evaluation with duckweed (Lemna minor L.) as a model plant	Pesticide biochemistry and physiology (2011), Vol. 99, No. 3, pp. 237-243	Use of glyphosate product in a study on lemna to assess the impact on biomass and Chlorophyll content of plants. This study was performed in Poland. The paper does not contribute to the renewal of glyphosate in the EU.	The formulation tested is RoundUp Ultra. The presence of POEA could not be confirmed. The study is potentially less relevant but supplementary based on RMS criteria.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary and assessment of relevance and reliability for Kielak E. et al. 2011 or a justification that Roundup Ultra contains POEA.
Koakoski G. et al.	2014	Agrichemicals chronically inhibit the cortisol response to stress in fish.	Chemosphere (2014), Vol. 112, pp. 85-91	End-points based on measured stress hormones are not relevant to an EU level Annex I ecotoxicology risk assessment for renewal.	RMS notes that survival was also investigated. Roundup Original was used. The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that states « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS.
Kondera E. et al.	2018	Effect of glyphosate-based herbicide on hematological and hemopoietic parameters in common carp (Cyprinus carpio L).	Fish physiology and biochemistry (2018), Vol. 44, No. 3, pp. 1011-1018	End-points presented are not relevant to an EU level risk assessment for glyphosate renewal in the EU.	The formulation used contains POEA. The study is not considered relevant by RMS.
Kostopoulou S. et al.	2020	Assessment of the effects of metribuzin, glyphosate, and their mixtures on the metabolism of the model plant Lemna minor L. applying metabolomics.	Chemosphere (2020), Vol. 239, pp. 124582	The paper describes a metabolomics approach to establish the impact of glyphosate alone and mixtures with metribuzin on the metabolome of lemna. Novel approach to biomarker detection is not considered in an EU level assessment.	The RMS does not agree with the applicant's reasoning and conclusion. Novel approach may still provide relevant data for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Kostopoulou S. et al. 2020

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Krynak K. L. *et al.*	2017	Rodeo™ Herbicide Negatively Affects Blanchard's Cricket Frogs (*Acris blanchardi*) Survival and Alters the Skin-Associated Bacterial Community.	Journal of Herpetology (2017), Vol. 51, No. 3, pp. 402-410	Uses a formulation that is not relevant to the EU renewal of Glyphosate (RODEO).	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable with restrictions. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Kumar M. S. A. et al.	2013	Toxic impacts of two organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp Streptocephalus dichotomus.	International Journal of Pharma and Bio Sciences (2013), Vol. 4, No. 2, pp. B-966	5.4.1 case b) Relevant but supplementary information: The test does not follow a recognised test guideline. There are no details on the test design used in the exposure part of the test, such as test media preparation and test vessels / replication details, and the water quality / environmental conditions during the exposure period. Nor are there any validity criteria stated, which are necessary to establish the acceptability of the study (eg. shrimp cyst hatching success and the percentage survival in the control group in both toxicity tests). There are no biological data presented to confirm the reported LC50 values. There is no rationale described justifying the duration of exposure. Details on the test substances used in the test are not presented and there is no analytical verification of test concentrations, so exposure levels cannot be verified. The study is considered unreliable.	The RMS agrees with the applicant's reasoning and conclusion.
Lacaze E. et al.	2010	Genotoxicity assessment in the amphipod Gammarus fossarum by use of the alkaline Comet assay	Mutation Research, Genetic Toxicology and Environmental Mutagenesis (2010), Vol. 700, No. 1-2, pp. 32-38	This study is the development of an assay. Endpoints cannot be used in the regulatory risk assessment of glyphosate.	The RMS agrees with the applicant's reasoning and conclusion.
Lajmanovich R. C. *et al.*	2011	Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase inhibitors.	Archives of environmental contamination and toxicology (2011), Vol. 60, No. 4, pp. 681-9	Compared toxicity to tadpoles exposed to a range of glyphosate products up to 240 mg ae/L for 48 hrs, enzyme activity was measured. Tadpoles collected from the wild (non-agricultural areas in Argentina, acclimated for 48 hrs). LC50 generated with very high concentrations tested.	The RMS considers the results on Roundup Ultra Max to be relevant and those on Infosato, C-K YUYOS FAV and Glifoglex less relevant but supplementary. The study is considered reliable with restrictions.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Lajmanovich R. C. *et al.*	2013	Individual and mixture toxicity of commercial formulations containing glyphosate, metsulfuron-methyl, bispyribac-sodium, and picloram on *Rhinella arenarum* tadpoles.	Water Air and Soil Pollution (2013), Vol. 224, No. 3, pp. Article No.: 1404	Formulation used in the testing is not the representative formulation for the Annex 1 renewal. Also difficult to relate the cellular and molecular level endpoints to an Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered relevant and reliable with restrictions. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Lallana M. d. C. et al.	2013	Determination of root length reduction (EC50) by a glyphosate formulation using lettuce and wheat as biological indicator species. Original Title: Determinacion de reduccion del crecimiento radical (CE50) por una formulacion de glifosato utilizando lechug	Revista de la Facultad de Ciencias Agrarias Universidad Nacional de Cuyo (2013), Vol. 45, No. 1, pp. 143-151	Endpoints presented were not generated using a test design that reflects use in the field and as such is not considered relevant / relatable to an EU level risk assessment for PPP Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Lance E. et al.	2016	Accumulation and detoxication responses of the gastropod *Lymnaea stagnalis* to single and combined exposures to natural (cyanobacteria) and anthropogenic (the herbicide RoundUp(®) Flash) stressors.	Aquatic toxicology (2016), Vol. 177, pp. 116-24	Molecular level results that are not relatable to an EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Lanctot C. *et al.*	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frog (*Lithobates sylvaticus*) tadpoles. II: agriculturally relevant exposures to Roundup WeatherMax® and Vision® under laboratory conditions.	Aquatic toxicology (2014), Vol. 154, pp. 291-303	The tested formulation contains POEA and is therefore not relevant to the MON 52276 representative formulation for the Annex I renewal.	We agree that the results on Roundup Vision are not relevant due to the presence of POEA. However, the results on Roundup Weather Max are considered less relevant but supplementary and reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Lanzarin G. A. B. et al.	2019	Dose-dependent effects of a glyphosate commercial formulation - Roundup(®) UltraMax - on the early zebrafish embryogenesis.	Chemosphere (2019), Vol. 223, pp. 514-522	Paper concerns a Roundup formulation that is not the representative formulation for the Annex I renewal.	The objective of this study was to investigate the lethal and sub-lethal developmental effects, neurotoxic potential and oxidative stress responses of zebrafish embryos to Roundup® Ultramax (RU) exposure. It is clearly stated in the study that Roundup® UltraMax contains ether amine ethoxylate (6 %). Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. Besides Avigliano L. et al., 2014 (other study) also stated that (based on a comparison between Roundup Ultramax and glyphosate at equivalent concentration), Roundup compounds other than glyphosate may be responsible for the effect of the formulation. The representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. Therefore RMS considers that this study is of low reliability. The study is considered not relevant.
Leccia F. et al.	2016	Disruption of the chemical communication of the European agrobiont ground-dwelling spider *Pardosa agrestis* by pesticides.	Journal of applied entomology (2016), Vol. 140, No. 8, pp. 609	5.4.1 case b) Relevant but supplementary information: Endpoints based on the impact of chemicals on spider pheromones are not used / required at EU level ecotoxicological regulatory risk assessments / glyphosate EU renewal.	The aim of the study was to investigate the sexual chemical communication of the beneficial agrobiont spider Pardosa agrestis and its disruption by the glyphosate-based herbicide Roundup Klasik. No sufficient information is available in the study report to demonstrate whether the tested formulation is

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					comparable with the representative formulation. The 3-h residues of Roundup Klasik (at recommended application rate) significantly disrupted the male ability of P. agrestis males to follow female cues deposited on dragline silk. Effect apparently transitory (no statistical difference with 48h old residues). RMS notes the absence of clear conceptual link between sexual chemical communication and the specific protection goals for Non-target arthropods. It is agreed that it may play a role in the population health, but such link is not immediate in conceptual terms and not quantifiable. The results of this study are not relevant for the regulatory risk assessment. RMs also notes that co-formulants are not stated. This study is not relevant for the regulatory risk assessment. Reliability was not assessed.
Liao L-H. et al.	2017	Behavioral responses of honey bees (Apis mellifera) to natural and synthetic xenobiotics in food.	Scientific reports (2017), Vol. 7, No. 1, pp. 15924	5.4.1 case b) Relevant but supplementary information: Presented data based on preference behaviour of honey bees cannot be directly related to an EU level ecotoxicological risk assessment - may possibly be used to support a lack of effects despite evidence being based upon preference.	This report states that bees displayed a preference at specific concentrations for glyphosate that may account for the frequency with which these pesticides occur as hive contaminants and suggests that they present a greater risk factor for honey bee health than previously suspected. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Liao L-H. et al. 2017

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Li Jia et al.	2017	Acute toxicity study of glyphosate and cyhalofop-butyl to Daphnia carinata.	Acta Prataculturae Sinica (2017), Vol. 26, No. 9, pp. 148	5.4.1 case b) Relevant but supplementary information: The herbicides evaluated in the study were a 41% glyphosate isopropylamine saline water agent (this formulation is not representative for the glyphosate EU renewal, the representative formulation is MON 52276). The study was not conducted according to GLP and the test substance source could not be verified.	Original report in Chinese, results are based on a document translated in english. The acute effects of glyphosate (in a formulation not identified) on the movement, survival, and phototaxis of Daphnia carinata were investigated. LC50 values after 24, 36, 48, 72 and 96 hours were 66.58, 58.13, 29.60, 18.83 and 12.33 mg/L, respectively. Regarding the effects on the activity ability the EC50 values after 24, 36, 48, 72 and 96 hours were 45.24, 42.49, 26.53, 17.14, and 11.58 mg/L respectively. No information on surfactants were provided. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. No analytical verification is available. Only LC50/EC50 are presented (no details on the results are provided). RMS considers that this study is not reliable and cannot be taken into account as critical information or the assessment of the active substance glyphosate itself. Less relevant but supplementary (uncertainty on the test item) and not reliable.
Lin JingWen et al.	2015	Toxic effect of glyphosate on seed germination and seedling growth of Chinese fir.	Acta Agriculturae Universitatis Jiangxiensis (2015), Vol. 37, No. 5, pp. 843	5.4.1 case b) Relevant but supplementary information: The study was not conducted to GLP, but it is well documented although no relevant guidelines have been followed. The authors state that the seed germination rate as	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information on woody species.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				well as the root length, stem length, leaf length and fresh weight of seedlings decreased significantly with the increase of glyphosate and the root length was more sensitive to glyphosate than other indexes. It was concluded that there is an inhibitory effect of glyphosate on Chinese fir seeds and seedlings, which led to antioxidant enzyme dysfunction, oxidative damage of cells and reduced chlorophyll synthesis. No analytical verification of the test item concentrations was performed, and the findings do not generate endpoints relevant to the regulatory risk assessment of glyphosate.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Lin JingWen et al. 2015
Li Jiao et al.	2010	Acute Toxicity of Eight Pesticides on the Development of Sea Urchin Embryos.	Asian Journal of Ecotoxicology (2010), Vol. 5, No. 2, pp. 255	The study of the toxicity to the sea urchin embryos, was not conducted or based on a relevant guideline. Test concentrations were from 0.1 to 50 mg/L of glyphosate technical. The relationship between EC50 and LogP values was the main discussion of the article In addition, it is a non-EU study conducted with local native species and influenced by different geo-climatic properties and land-uses and agricultural practices. The study does not present any data which can be used in the ecotoxicological regulatory risk assessment / glyphosate EU renewal.	Original report in Chinese, results are based on a document translated in english. Acute toxic effects of glyphosate on the embryo development of the sea urchin Strongylocentrotus intermedius were investigated. Acute toxicity of glyphosate causes gradually decrease of the EC50 with the development of the sea urchin (Strongylocentrotus intermedius) embryo with a lowest EC50 of 3.99 mg/L at the last stage, the 4-arm pluteus (52 h development). It was not based on a relevant guideline (no guideline available). Only EC50 is available, no biological data were presented. No NOEC was derived. RMS considers that this study is of low relevance as it does not present

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					any data which can be used in the ecotoxicological regulatory risk assessment. Less relevant but supplementary (endpoint not relatable to the risk assessment scheme) and not reliable (no data available in the report).
Li M. et al.	2017	Metabolic profiling of goldfish (*Carassius auratis*) after long-term glyphosate-based herbicide exposure.	Aquatic toxicology (2017), Vol. 188, pp. 159- 169	Metabolomic approaches to assessing the fate of pesticides in organisms looks specifically at cellular and molecular level based endpoints that are not used in the EU level ecotoxicology risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Li P-L. et al.	2015	Response of *Nitzschia amplectens* in growth and kinestate to glyphosate original powder	Nongyao (2015), Vol. 54, No. 2, pp. 108-111	Based on Roundup Original which contains POEA which is not relevant at EU level for MON 52276 renewal. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the renewal.	The original report is in Chinese. The translation available did not mention Roundup Original as test item but Glyphosate original Powder. The relevance status is unclear. Dat gap: Provide a summary and assessment of relevance and reliability of the paper of Li P-L. et al. 2015 or justify that the test was indeed performed with a formulation containing POEA.
Li Q. et al.	2013	Effects of herbicides application on allelopathic potential of Eupatorium catarium.	Allelopathy Journal (2013), Vol. 31, No. 1, pp. 139-146	Observations caused by mixture of compounds / potentially causal factors and thus not attributable to a substance of concern (e.g. mixture toxicity).	Combined exposure and toxicity. Thus RMS agrees with the applicant's reasoning and conclusion.
Li Y. et al.	2019	Acute exposure of glyphosate-based herbicide induced damages on common carp organs via heat shock proteins-related immune response and oxidative stress	Toxin Reviews (2019), Ahead of Print, https://doi.org/10.1080/15569543.2019.1621903	Formulation is not the representative formulation for the annex I renewal of glyphosate. As the identity of the powder and the form in which it was supplied (salt type, to establish acid equivalence content) cannot be confirmed. Co-formulants are also unknown.	Study may be considered as less relevant but supplementary based on RMS criteria. Assessment of reliability requested. Data gap : Provide a summary and assessment of relevance and reliability of the paper of Li Y. et al. 2019.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Lipok J. et al.	2010	The toxicity of Roundup® 360 SL formulation and its main constituents: glyphosate and isopropylamine towards non-target water photoautotrophs.	Ecotoxicology and environmental safety (2010), Vol. 73, No. 7, pp. 1681-8	Uses glyphopsate product, study looking at impact on marine microbial (algae) communities (14 d old log-phase cultures used), exposed up to 3mM of GLY. EC50 generated. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	Study may be considered as less relevant but supplementary based on RMS criteria. Assessment of reliability requested. Data gap : Provide a summary and assessment of relevance and reliability of the paper of Lipok J. et al. 2010.
Liu C. et al.	2012	Size-controlled preparation of hollow silica spheres and glyphosate release	Transactions of Nonferrous Metals Society of China (2012), Vol. 22, No. 5, pp. 1161-1168	This paper relates to the development of a silica capsule. Glyphosate is mentioned as the example chemical that demonstrates increased release rate with thinning of the capsule wall. Not relevant for 2022 ecotox renewal risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Liu Xiao-wei et al.	2012	Toxicological effect of paraquat and glyphosate on cladoceran Moina macrocopa.	Shengtaixue Zazhi (2012), Vol. 31, No. 8, pp. 1984	5.4.1 case b) Relevant but supplementary information: The conclusions of the study are unclear based on several factors including the impact of the density of the algal food source and the temperature of the test media.	Original report in Chinese, results are based on a document translated in english. It was not based on a relevant guideline (no guideline available). Only LC50 is available, no biological data were presented. No NOEC was derived. This study is not adequately described. There were no validity criteria stated and no analytical verification of exposure concentrations was undertaken. Inconsistencies are noted in the translated report e.g. LC50 reported in the summary does not correspond to the one given in the results chapter. The study is relevant but not reliable
Lo C-C.	2010	Effect of pesticides on soil microbial community.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2010), Vol. 45, No. 5, pp.	Conducted in China. Review of toxicity studies to look at the effect of glyphosate and other chemistry to soil microflora. As it's a review of other data it doesn't bring any specific endpoint to the Regulatory risk assessment of glyphosate renewal.	No critical data was found (for glyphosate) in this report. No relevant information can be used for the risk assessment.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
			348-59		
Lopes da Silva E. T. et al.	2016	Lethal concentration of glyphosate for juveniles of curimata-pacu. Original title: concentracao letal do glifosato para juvenis De curimata-pacu.	Boletim Do Instituto De Pesca (2016), Vol. 42, No. 4, pp. 759-764	Concerns a formulation of Glyphosate (ATANOR®) that is not the representative formulation for the Annex I renewal.	The objective of this study was to determine the LC50 of the herbicide glyphosate (LC50) for the curimatã-pacu fish, Prochilodus argenteus. 240 juvenile curimatã-pacu fish (standard length of 6.4 ± 8.47 cm; and weight of 8.98 ± 3.91 g) were used. The LC50 of glyphosate at 48, 72 and 96 h were of 20.88, 19.91 and 19.09 mg/L, respectively. This study may indicate higher sensitivity of this species but the co-formulants in Atanor are not stated and may have impacted the toxicity of the product. The representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. Moreover, no biological data and no analytical verification are presented in the report. No control data is reported. The report also states that from the minimum concentration of glyphosate at 0.5 mg L-1, all juveniles were initially agitated, followed by an increase in the beat of the operculum and later they showed to be swimming in erratic fashion and with lethargic behaviour. Progressive turbidity of the water was observed and also the presence of air bubbles, leaving it with a foamy appearance. This questions the conditions of the fish in the test. The study is less relevant but supplementary (due to the different formulation that was tested) and not reliable.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Lopes F. M. et al.	2018	Toxicity induced by glyphosate and glyphosate-based herbicides in the zebrafish hepatocyte cell line (ZF-L).	Ecotoxicology and environmental safety (2018), Vol. 162, pp. 201-207	The formulated product used in the test contains MON 2139 which contains POEA (MON0818). Therefore findings are not relevant to the EU level renewal as are not representative formulation for the Annex I renewal.	The study also presented the results for glyphosate alone. The authors concluded that only in the commercial formulation but also glyphosate alone, since both can induce damage in the ZF-L cell line primarily through the induction of apoptosis. Further investigation may be needed. The absence of population relevance might be necessary. Dat gap : Provide a summary and an assessment of relevance and reliability of the paper of Lopes F. M. et al. 2018.
Lopes F. M. et al.	2017	Glyphosate Adversely Affects Danio rerio Males: Acetylcholinesterase Modulation and Oxidative Stress.	Zebrafish (2017), Vol. 14, No. 2, pp. 97-105	Endpoints not relatable to an EU level ecotoxicology risk assessment for Annex I renewal.	Glyphosate exposure caused an imbalance in the oxidative status in Danio rerio males exposed to 5 or 10mg/L of glyphosate and altered the cholinergic system in a tissue-dependent manner. No quantitative link can be made between these effects and potential effect at population level. These data are not relatable to the risk assessment.
Louch J. et al.	2017	Potential risks to freshwater aquatic organisms following a silvicultural application of herbicides in Oregon's Coast Range.	Integrated environmental assessment and management (2017), Vol. 13, No. 2, pp. 396-409	This is a specific non-EU monitoring study that cannot be related to an EU level ecotoxicology risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Lozano V. L. et al.	2018	Effects of glyphosate and 2,4-D mixture on freshwater phytoplankton and periphyton communities: a microcosms approach	Ecotoxicology and Environmental Safety (2018), Vol. 148, pp. 1010-1019	The focus of the study was on phytoplankton and periphyton communities. However, no information on the source and history of the phytoplankton and periphyton communities are given. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment. (The glyphosate formulation Glifosato Atanor® was used in the	The study is considered less relevant but supplementary by RMS (see criteria in appendix to Volume 3 CA B.9 on literature data on ecotoxicology). Assessment of reliability necessary. Data gap : provide a summary and assessment of relevance and reliability

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				microcosm study). In addition, no regulatory useful endpoint was derived.	for the paper of Lozano V. L. et al. 2018.
Lugowska K.	2018	The effects of Roundup on gametes and early development of common carp (Cyprinus carpio L)	Fish physiology and biochemistry (2018), Vol. 44,No. 4, pp. 1109	5.4.1 case b) Relevant but supplementary information: The tested formulation is not the representative formulation for the glyphosate EU renewal (the representative formulation is MON 52276).	This study investigates parameters of interest for the risk assessment. Full summary is therefore reported despite the drawbacks of the study. The effects of Roundup Ultra 170 SL Transorb on embryos of common carp (Cyprinus carpio L) were investigated with wide range of concentrations (0.0, 0.1, 0.5, 2.0, 5.0, 10.0, 20.0, and 50.0 mg/L). Effects of Roundup Ultra 170 SL Transorb on common carp gametes were observed. Herbicide reduced swelling of eggs (but the effect was not concentration-related), while sperm showed low sensitivity to Roundup Ultra 170 SL Transorb (time of spermatozoa motility was reduced in a significant way only at 20 mg a.i./l, and at remaining concentrations, only a slight tendency was observed). RMS notes the absence of clear conceptual link between swelling of eggs, sperm motility and the specific protection goals for fish. It is agreed that it may play a role in the population health, but such link is not immediate in conceptual terms and not quantifiable. The results on these parameters are not relevant for the risk assessment. During the embryonic development, Roundup caused a decrease of common carp embryonic survival (and the effect was concentration-

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					related); Survival of embryos gradually decreased during the embryonic development and was significantly lower at each stage in groups exposed to Roundup compared to the control. During the embryogenesis, three types of embryo body malformation were observed: yolk sac edema, spine curvature, and shortening of body, but their frequencies were not associated with the presence or concentration of herbicide. Roundup affected quality of newly hatched larvae of common carp by increasing their mortality. No effect of herbicide on percentage of deformed larvae was observed but larvae hatched in water with Roundup tended to show more complex anomalies compared to those from the control. NOECembryonic survival < 0.1 mg glyphosate/L (lowest tested concentration) This study doesn't follow a specific guideline (no validity criteria available for these parameters but the performance of control and the dose-effect relationship provides some reliability to the results). No analytical verification of test concentrations is reported. Only graphics are available (no biological data presented in the report).

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Roundup Ultra 170 SL Transorb was used. RMS notes that co-formulants are not stated. The study is less relevant but supplementary (difference of formulation tested with representative formulation not clear) and not reliable.
Lu Li-li et al.	2010	Effects of glyphosate on the growth and development of Agasicles hygrophila	Huanan Nongye Daxue Xuebao (2010), Vol. 31, pp. 22	5.4.1 case b) Relevant but supplementary information: The test substance is 41% glyphosate IPA salt. The study on Agasicles hygrophila was not conducted or based on a relevant NTA guideline.	The experiment was designed to assess the effects of glyphosate (in a formulation not defined) on the development of Agasicles hygrophila (a weed flea beetle species). There was no difference in the hatching rate of eggs between the glyphosate treatment and the control group at all concentrations tested. However, the survival rates of larvae and adults treated by glyphosate were significantly lower than those in the control group. The survival rates of larvae and adults in the control group were 1.59- and 1.31-fold of that in the treated group with 2.05 g/L glyphosate, respectively. This study was not conducted or based on a relevant NTA guideline. RMS questions the relevance of the exposure. A monolayer filter paper was placed in the petri dish and was treated with glyphosate solutions wetting filter paper. Results are all expressed in terms of concentrations but the volumes applied in Petri dishes are not explained. Then the relevance of the exposures in this study is questionable. The results are not

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					relatable to EU regulatory risk assessment. The test item is not well identified. This study is considered not relevant by RMS.
Lyons M. et al.	2018	Effects of 4-nonylphenol and formulations of five pesticides: cypermethrin, deltamethrin, glyphosate, imidacloprid and mancozeb on growth of Atlantic salmon (Salmo salar L.) during parr-smolt transformation.	Canadian Technical Report of Fisheries and Aquatic Sciences (2018), Vol. 3265, pp. 1-42	Fish exposed to a glyphosate formulation that is not the representative formulation for the Annex I renewal.	The aims of this study were to examine the effects of a glyphosate formulation Roundup WeatherMax (and 5 other products containing other substances) on growth performance of Atlantic salmon smolts. Sub-lethal cellular effects using biochemical endpoints were also investigated. This study did not follow a standardized guideline. The growth effects observed on fish exposed to glyphosate formulation at an environmentally realistic concentration of 65 µg/L (measured 32 µg/L ± 5) evoke particular concern for the survival of wild Atlantic salmon smolts. Mean Na+, K+-ATPase activity showed decreases in treated fish but the differences were not significant. RMS considers the growth rate as the main parameter of interest in this study. While acknowledging that a lower growth may have temporarily affected the smolts (significant at lowest concentration), RMS notes that this was not concentration related (no significant effect at the highest concentration 3000 µg/L).

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					No biological data was presented, only graphs. It is difficult to explain the high difference of growth rates between the control groups used for the different experiments (control groups for cypermethrin experiments were very high compared to those of glyphosate experiments). No biological observations are reported. Roundup WeatherMax was used. The presence of toxic surfactants were not precised. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product (as also mentioned by the study authors concerning the formulation they used). The representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. Therefore RMS considers that this study is less relevant but supplementary (due to the different formulation tested) and not reliable. The acute toxicity of glyphosate on common carp was first determined; then, the contents of interferon- γ (IFN-γ), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) and histopathological alterations in the liver, kidneys, and spleen of common carp exposed to 52.08 or 104.15 mg/L

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					of glyphosate for 168 h were also determined and evaluated. - Glyphosate has low toxicity on common carp (96 h LC50 = 520.77 mg/L). - Glyphosate-exposure alters the contents of cytokines. - Glyphosate caused histopathological damage to common carp. - Glyphosate has immunotoxic effects on common carp. Sublethal effects were investigated at concentrations of 52.08 and 104.15 mg/L of glyphosate (for 168 h). These concentrations are far from those expected under realistic conditions of use. The formulation tested contains 50% glyphosate and is a soluble powder. The representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. Total hardness was of 340 mg/L, no analytical verification is available, so potential interaction with ions in the environmental conditions of this study are unknown. In OECD 203, the recommended hardness for common carp is 40 to 250 mg/L (preferably less than <180). Fish were rather big, RMS doubts their sensitivity. Overall, despite statistically significant, the effects do not seem concentration

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					related. The results presented graphically only. The study is less relevant but supplementary (due to the uncertainty around the test item) and not reliable for risk assessment purpose.
Ma J. et al.	2015	Alteration in the cytokine levels and histopathological damage in common carp induced by glyphosate.	Chemosphere (2015), Vol. 128, pp. 293-8	Endpoints not relatable to an EU level risk assessment for Annex I renewal.	The acute toxicity of glyphosate on common carp was first determined; then, the contents of interferon- γ (IFN-γ), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) and histopathological alterations in the liver, kidneys, and spleen of common carp exposed to 52.08 or 104.15 mg/L of glyphosate for 168 h were also determined and evaluated. - Glyphosate has low toxicity on common carp (96 h LC50 = 520.77 mg/L). - Glyphosate-exposure alters the contents of cytokines. - Glyphosate caused histopathological damage to common carp. - Glyphosate has immunotoxic effects on common carp. Sublethal effects were investigated at concentrations of 52.08 and 104.15 mg/L of glyphosate (for 168 h). These concentrations are far from those expected under realistic conditions of use. The formulation tested contains 50% glyphosate and is a soluble powder. The representativness of the results to

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					assess the toxicity of glyphosate as formulated MON52276 is questionable. Total hardness was of 340 mg/L, no analytical verification is available, so potential interaction with ions in the environmental conditions of this study are unknown. In OECD 203, the recommended hardness for common carp is 40 to 250 mg/L (preferably less than <180). Fish were rather big, RMS doubts their sensitivity. Overall, despite statistically significant, the effects do not seem concentration related. The results presented graphically only. The study is less relevant but supplementary (due to the uncertainty around the test item) and not reliable for risk assessment purpose.
Ma J. et al.	2015	Immunological and histopathological responses of the kidney of common carp (Cyprinus carpio L.) sublethally exposed to glyphosate.	Environmental toxicology and pharmacology (2015), Vol. 39, No. 1, pp. 1-8	Molecular level results that are not relatable to an EU level ecotoxicology risk assessment.	Sublethal effects were investigated at concentrations of 52.08 and 104.15 mg/L of glyphosate (for 168 h). These concentrations are far from those expected under realistic conditions of use. The formulation tested contains 50% glyphosate and is a soluble powder. The representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. Total hardness was of 340 mg/L, no analytical verification is available, so potential interaction with ions in the environmental conditions of this study are unknown. In OECD 203, the recommended hardness for common

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					carp is 40 to 250 mg/L (preferably less than <180). The study is less relevant but supplementary (due to the uncertainty around the test item) and not reliable enough for risk assessment purpose.
Ma J. et al.	2019	Biochemical and molecular impacts of glyphosate-based herbicide on the gills of common carp.	Environmental pollution (2019), Vol. 252, No. Pt B, pp. 1288-1300	Paper discusses biochemical and molecular impacts that are not relatable to an EU level RA for Annex I renewal	The tested formulation is indicated to contain POEA. Thus the study is not relevant.
Magano D. A. et al.	2013	Side-effects of herbicides applied in soybean Trichogramma pretiosum. Efeitos secundarios de herbicidas aplicados em soja sobre Trichogramma pretiosum.	Pesquisa Agropecuaria Gaucha (2013), Vol. 19, No. 1/2, pp. 69-80	The formulations used that contain glyphosate were Glyphosate Atanor and Roundup Original. The Roundup formulation contains POEA and is therefore not relevant for the EU. Concerning ATANOR, this is not the representative formulation and it is difficult to relate the observed effects with the ecotoxicology risk assessment for EU Annex I renewal of MON 52276.	The results with Atanor formulation may be considered as less relevant but supplementary based on RMS criteria (see appendix to volume 3 CA B.9 related to letrature data on ecotoxicology). Furtehr inverstigation is needed. Data gap :Provide summary and assessment of relevance and reliability for the paper of Magano D. A. et al. 2013.
Magbanua F. S. et al.	2013	Understanding the combined influence of fine sediment and glyphosate herbicide on stream periphyton communities.	Water research (2013), Vol. 47, No. 14, pp. 5110-20	This study investigated the combination of sediment and glyphosate effects on an mesocosm community. Results not relatable to an EU level ecotoxicological risk assessment.	In this study, it is clearly stated by the study authors that the formulation included the surfactant polyethoxylated tallow amine (POEA). Since August 2016, POEA is not authorized in plant protection products containing glyphosate (European Commission). Therefore it is not possible to discriminate between glyphosate and POEA. The study is considered not relevant by RMS.
Magbanua F. S. et al.	2013	Individual and combined effects of fine sediment and the herbicide glyphosate on benthic macroinvertebrates and stream	Freshwater biology (2013), Vol. 58, No. 8, pp. 1729-1744	Paper describes a specific multiple mesocosm study conducted in New Zealand using an undefined source of glyphosate.	Since August 2016, POEA is not authorized in plant protection products containing glyphosate (European Commission). Therefore it

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		ecosystem function			is not possible to discriminate between glyphosate and POEA. The study is considered not relevant by RMS.
Malecot M. et al.	2013	Specific proteomic response of Unio pictorum mussel to a mixture of glyphosate and microcystin-LR.	Journal of proteome research (2013), Vol. 12, No. 11, pp. 5281-92	Observed findings are not relatable to an EU level ecotoxicological risk assessment for Annex I purposes.	The RMS agrees with the applicant's reasoning and conclusion.
Mandl K. et al.	2018	Effects of Glyphosate-, Glufosinate- and Flazasulfuron-Based Herbicides on Soil Microorganisms in a Vineyard.	Bulletin of environmental contamination and toxicology (2018), Vol. 101, No. 5, pp. 562-569	The product used was Roundup Powerflex, which is based on MON 79351 that contains 47.6% acid equivalence, and not MON 52276. Endpoints based on bacterial CFUs are difficult to relate to an ecotoxicological Annex I risk assessment. The work was also conducted in a vineyard that had a history of other pesticides being used. As identified by the Author, this cannot be excluded as having influenced the findings.	Results on vineyard soil for which the presence of several chemical could not be excluded. Thus it could not be used for renewal of approval of glyphosate.
Maria M. A. et al.	2018	Evaluation of glyphosate effect concentration to control Eichhornia crassipes and Salvinia sp. Avaliacao da concentracao de efeito do glifosato para controle de Eichhornia crassipes e Salvinia sp.	Engenharia Sanitaria e Ambiental (2018), Vol. 23, No. 5, pp. 881-889	On translated paper review, it is apparent that the study was conducted with a formulation (Roundup Original) that contains POEA - uncertain if observed effects were due to product or down to the action of POEA. POEA is not in the Annex I representative formulation and therefore these findings are not relevant to the ecotoxicology risk assessment for renewal.	The RMS accepted the applicant's reasoning and conclusion. Other papers using Roundup original indicated that it contains POEA (Sanchez et al 2017 : it was stated that states « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants."). Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS.
Marusca T.	2017	Oversowing or resowing of subalpine grassland appointed	Romanian Journal of Grassland and Forage	Study describes ecological succession and not specific effects of glyphosate on NTOs, therefore	The RMS agreed with the applicant's reasoning and conclusion.

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		after the dynamics of floristic composition.	Crops (2017), No. 15, pp. 45-55	not relevant to EU level Annex I ecotoxicology risk assessment.	
Mateos-Naranjo E. et al.	2013	Effects of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non- target species Bolboschoenus maritimus.	Chemosphere (2013), Vol. 93, No. 10, pp. 2631-8	End-points not considered relevant to an EU level risk assessment (ecotoxicology) for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Mateos-Naranjo E. et al. 2013
Matozzo V. et al.	2018	Ecotoxicological risk assessment for the herbicide glyphosate to non-target aquatic species: A case study with the mussel *Mytilus galloprovincialis*.	Environmental pollution (2018), Vol. 233, pp. 623-632	No control data are presented. The concept of up and down- regulation of genes following exposure to glyphosate within the context of a risk assessment is not relatable to the EU renewal. The purity of the test substance is not presented so dosing cannot be confirmed. The environmental conditions of the exposure phase are not presented other than salinity and temperature. No positive control included.	The RMS agrees with the applicant's reasoning and conclusion.
Matozzo V. et al.	2018	Effects of aminomethylphosphonic acid, the main breakdown product of glyphosate, on cellular and biochemical parameters of the mussel Mytilus galloprovincialis	Fish & shellfish immunology (2018), Vol. 83, pp. 321-329	Cellular level parameters discussed in paper, with endpoints that are not relevant to an Annex I renewal from ecotoxicology perspective.	The RMS agrees with the applicant's reasoning and conclusion.
Matozzo V. et al.	2019	Glyphosate affects haemocyte parameters in the clam *Ruditapes philippinarum.*	Marine environmental research (2019), Vol. 146, pp. 66-70	Paper contains data that cannot be related to an EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Matozzo V. et al.	2019	Ecotoxicological hazard of a mixture of glyphosate and aminomethylphosphonic acid to the mussel Mytilus galloprovincialis (Lamarck 1819).	Scientific reports (2019), Vol. 9, No. 1, pp. 14302	End-points based on enzyme levels cannot be related to the EU level Annex I risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.

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McNally S. R. et al.	2017	Herbicide application during pasture renewal initially increases root turnover and carbon input to soil in perennial ryegrass and white clover pasture	Plant and soil (2017), Vol. 412, No. 1-2, pp. 133-142	Looks at root turnover as a means of sequestering carbon into soils. No specific endpoints useable in ecotoxicology EU level risk assessment for EU Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
McVey K. A. et al.	2016	Exposure of C. elegans eggs to a glyphosate-containing herbicide leads to abnormal neuronal morphology.	Neurotoxicology and teratology (2016), Vol. 55, pp. 23-31	The article does not report results, which can be used for risk assessment and information is insufficient to transfer values into such determinants.	The RMS agrees with the applicant's reasoning and conclusion.
Medeiros E. V. d. et al.	2014	Impact of glyphosate on microbial attributes of soil planted with two species of passion fruit.	Revista Caatinga (2014), Vol. 27, No. 1, pp. 1-8	Non-EU soil based comparative experiment to establish the impact of glyphosate on bacterial populations in soil for two different species of passion fruit in Brazil. The test design was described without specific detail on the amount of glyphosate being applied so any impacts could not be related to exposure. Therefore findings cannot be related to an EU level risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Mekhed O. B. et al.	2013	Impact of Water Pollution by Herbicides Zenkor and Roundup on Metabolism in Liver of Fishes of the Fam. Cyprinidae.	Hydrobiological Journal (2013), Vol. 49, No. 5, pp. 74-80	Molecular level results that are not relatable to an EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Menendez- Helman R. J. et al.	2015	Subcellular energy balance of Odontesthes bonariensis exposed to a glyphosate-based herbicide.	Ecotoxicology and environmental safety (2015), Vol. 114, pp. 157-63	Molecular level results that are not relatable to an EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Menezes C. W. G. et al.	2012	Reproductive and toxicological impacts of herbicides used in Eucalyptus culture in Brazil on the parasitoid Palmistichus elaeisis (Hymenoptera: Eulophidae)	Weed research (2012), Vol. 52, No. 6, pp. 520-525	Article not investigating properties of the active substance glyphosate. The articles does not cover any data requirement under EC Regulation 1107/2009.	The RMS agrees with the applicant's reasoning and conclusion.
Mensah P. et al.	2012	Using growth measures in the freshwater shrimp Caridina nilotica as biomarkers of	Physics and Chemistry of the Earth, Parts A/B/C	The formulation is based on MON 2139, which contains MON 0818 (that includes POEA) which is not relevant for the EU representative	The RMS agrees with the applicant's reasoning and conclusion.

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		Roundup registered pollution of South African freshwater systems	(2012), Vol. 50, pp. 262-268	formulation. The confounding effects of POEA on the results cannot be excluded as no glyphosate technical grade only treatments were included, therefore findings are considered relevant to EU level ecotoxicology risk assessment for Annex I renewal.	
Mensah P. K. et al.	2012	Acetylcholinesterase activity in the freshwater shrimp Caridina nilotica as a biomarker of Roundup (R) herbicide pollution of freshwater systems in South Africa.	Water Science and Technology (2012), Vol. 66, No. 2, pp. 402-408	Formulation used in the study contains POEA that is not relevant to the EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Meshkini S. et al.	2019	The acute and chronic effect of Roundup herbicide on histopathology and enzymatic antioxidant system of Oncorhynchus mykiss.	International Journal of Environmental Science and Technology (2019), Vol. 16, No. 11, pp. 6847-6856	Roundup was used which contains POEA. This is not the representative formulation for the Annex I renewal.	Other studies indicated the Roundup contains POEA. The fact that Roundup PRO indeed contains POEA should be justified. Data gap : Provide a justification that Roundup PRO used in Meshkini S. et al. 2019 contains POEA. Otherwise relevance of reliability of the paper will have to be assessed.
Milan M. et al.	2018	Ecotoxicological effects of the herbicide glyphosate in non-target aquatic species: Transcriptional responses in the mussel Mytilus galloprovincialis.	Environmental pollution (2018), Vol. 237, pp. 442-451	No control data are presented. The concept of up and down- regulation of genes following exposure to glyphosate within the context of a risk assessment is not relatable to the EU renewal. The purity of the test substance is not presented so dosing cannot be confirmed. The environmental conditions of the exposure phase are not presented other than salinity and temperature. No positive control included.	The RMS agrees with the applicant's reasoning and conclusion.
Mohamed I. A-w. et al.	2016	Unique efficacy of certain novel herbicides against Culex pipiens (Diptera: Culicidae) mosquito under laboratory conditions	Advances in Environmental Biology (2016), Vol. 10, No. 8, pp. 104	5.4.1 case b) Relevant but supplementary information: Important information is missing in the material and methods section. The preparation and application of the test solutions as well as the tested concentration range were not reported. The test items were not adequately specified. It is not clear whether the test	The RMS agrees with the applicant's reasoning and conclusion. RMS notes that toxicity on *Culex pipiens* was very low.

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				concentrations refer to the product or to the active substance. Moreover one active ingredient is given as glyphosate isopropylamine which should be formulated as a salt resulting in test concentrations as acid equivalents. In addition, the biological results of the test were not sufficiently stated. No mortality data for the test concentrations nor for the controls was given to evaluate the results. Furthermore, there was no analytical verification of test concentrations reported. The study is not to a guideline and is not GLP. The study is considered unreliable.	
Mona M. H. et al.	2013	Evaluation of cytotoxic effects of atrazine and glyphosate herbicides on *Biomphalaria glabrata* snails.	Journal of Basic and Applied Zoology (2013), Vol. 66, No. 2, pp. 68-75	Paper discusses a RAPD-PCR technique for detecting genotoxic damage. Formulation tested (Herfosat, Egypt; not characterized/described further). The data are not relatable to an EU level risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Mondal S. et al.	2017	Phytotoxicity of glyphosate in the germination of Pisum sativum and its effect on germinated seedlings.	Environmental health and toxicology (2017), Vol. 32, pp. e2017011	Test design not relevant to EU level ecotoxicological risk assessment for Annex I renewal.	In this study, 20 seeds were placed on filter paper inside a sterilized 15 cm Petri plate for seed germination and seedling growth. In each Petri plate 20 mL of glyphosate (Roundup, Marysville, OH, USA) at different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0 mg/ L doses. Germination and seedling growth were recorded up to 14 days at intervals of 24 hours. These concentrations were considered environmentally realistic by the study authors, but this is not explained. RMS is of the opinion that such concentrations are low if it intents to mimic the concentration in the spray. It is not known if this concentration aims to mimic porevater in soil.

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					Such endpoint cannot be related to the risk assessment for the non-target plants (endpoints being expressed as grammes per ha in the standard EU risk assessment). Besides, the influence of potential toxic surfactants has not been reported. The formulation is not described and the representativness of the results to assess the toxicity of glyphosate as formulated MON52276 is questionable. The study is considered not relevant by RMS for risk assessment purpose (due to the uncertainty on the test item and the exposure which is not relatable to the risk assessment).
Monquero P. A. et al.	2016	Initial growth of tree species under herbicide drift.	Revista de Ciencias Agrarias / Amazonian Journal of Agricultural and Environmental Sciences (2016), Vol. 59, No. 2, pp. 162-172	Direct application to trees is not a proposed use of glyphosate and such end-points are not used in the EE level risk assessment for glyphosate renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Monte T. C. C. et al.	2019	Changes in hemocytes of Biomphalaria glabrata infected with Echinostoma paraensei and exposed to glyphosate-based herbicide.	Journal of invertebrate pathology (2019), Vol. 160, pp. 67-75	Relates to snails being exposure to a formulation of glyphosate that is not the representative formulation at Annex I for the EU.	The formulation that was used (Roundup Original) contains POEA (as stated in the report). The study is not relevant.
Moreira L. F. et al.	2019	Modulation of the multixenobiotic resistance mechanism in Danio rerio hepatocyte culture (ZF-L) after exposure to glyphosate and Roundup (R).	Chemosphere (2019), Vol. 228, pp. 159-165	Epigenetic biomarkers are indicators of the presence of a chemical or mixture of chemicals in the environment. They are not indicators of toxicity. The endpoints presented are not relatable to the ecotoxicological risk assessment required for Annex I renewal in the EU.	No link between modulation of the multixenobiotic resistance mechanism in Danio rerio hepatocyte culture (ZF-L) and impact on population could be made. Thus the RMS accepted the applicant's reasoning and conclusion.

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Morgan M. A. et al.	2019	Evaluating sub-lethal stress from Roundup (R) exposure in *Artemia franciscana* using H-1 NMR and GC- MS.	Aquatic Toxicology (2019), Vol. 212, pp. 77-87	Formulation used contains POEA - not relevant therefore to EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Morris A. et al.	2016	Effect of two commercial herbicides on life history traits of a human disease vector, Aedes aegypti, in the laboratory setting.	Ecotoxicology (2016), Vol. 25, No. 5, pp. 863-70	No relevant information on metabolism / residues / background levels of glyphosate. Epidemiology, effect of glyphosate on Aedes aegypti.	The RMS agrees with the applicant's reasoning and conclusion.
Motta E. V. S. et al.	2018	Glyphosate perturbs the gut microbiota of honey bees.	Proceedings of the National Academy of Sciences of the United States of America (2018), Vol. 115, No. 41, pp. 10305	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This papers describes exposure of bees to glyphosate and its impact on gut microbiota.	This study states that glyphosate had some effect on honeybee microbiota. RMS notes the absence of clear conceptual link between effects on the honeybee microbiota and the specific protection goals for bees (SPG). It is agreed that it may play a role in the colony/population health, but such link is not immediate in conceptual terms and not quantifiable. The only bee mortality reported were related to an exposure to an opportunistic bacterial pathogen Serratia marcescens kz19 (with and without glyphosate). This study states that glyphosate reduces the protective effect of the gut microbiota against opportunistic pathogens. The effect of such synergistic effects are however not covered by the current risk assessment scheme. Several experiments were performed in the study and the details of those studies were mainly presented in the Supplementary Information (not provided by the applicant but could be retrieved by RMS).

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					Results Hive experiments Two hive experiments were performed, in autumn and spring. In each experiment, the same procedure was followed: 2000 adult bees were collected from a single hive, separated into 3 groups (control, 5 and 10 mg glyphosate/L), and placed into cup cages (40 bees per cup cage, totaling 16 cup cages per group). The bees were exposed to glyphosate during 5 days. Then 15 bees from each group were sampled (Day 0), and 600 bees from each group were returned to the hive (it is not reported how these were chosen, nor what was done with the other 1385 bees). At Day 3 post exposure (Day 3), 15 marked bees from each group were sampled from the hive. Fewer than 20% of returned bees were recovered from each group at Day 3. Relative and absolute abundances of gut bacteria were assessed. The exposure levels chosen in the hive experiment (5 and 10 mg glyphosate/L) are claimed to mimic those expected in fields, i.e. 1.4 – 7.6 mg glyphosate/L (a reference is made to another article). The relevance of these exposure levels is not established. In the first hive experiment, at d0 glyphosate exposure had little effect on the bee gut microbiome size (total bacteria number). The authors claim that the effects of glyphosate exposure

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					on the bee gut microbiome were more prominent at day 3, after treated bees were returned to the hive. However, although the effects of G-5 treatment were statistically significant relative to the control, the abundance of bacteria in the control and the G-10 treatment were similar at d3. Therefore, the biological relevance of the results is not fully justified at d3. In the second hive experiment, significant differences were not found between the control and the treatments at either d0 or d3 (see graphic below). Numbers of total bacterial 16S rDNA copies for control (C) and glyphosate-treated (G-5 and G-10) bees at post-treatment Days 0 and 3 (n = 13 for each group and time point) in the second hive experiment. The report states that absolute abundances of Lactobacillus Firm-4 (among others) were decreased in G-5 and G-10. However RMS notes that relative and absolute abundances of Lactobacillus Firm-4 increased in control group from d0 to d3. This indicates that these parameters were not steady and a shift may not be explained by dose treatment only. At d0, the relative and absolute abundances of the core species, S. alvi, were significantly lower in the G-10 group at d0, while this reduction was not obvious at d3 (Fig 1, A), this was not explained.

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					In the second hive experiment, a significant reduction in absolute abundance of S. alvi was observed at d0 and d3 in the G-5 and G-10 treatment (although no clear concentration response could be established). (see graphic below). RMS notes that absolute abundance was not homogeneous among bees in different groups (including control at Day 3) and the number of bees was low (13). Numbers of 16S rDNA copies for S. alvi for control (C) and glyphosate-treated (G-5 and G-10) bees at post-treatment Days 0 and 3 (n = 13 for each group and time point) in the second hive experiment. Several drawbacks in this study are noted: - the sample size was 15 bees per treatment (2 sampling times = 30 bees), which is relatively low to provide an understanding of natural background variability versus actual effects, - bees from only 2 hives were sampled (spring and autumn), - the rearing conditions of bees were not reported, which might have influence on the results and is of importance to judge the health of bees, - all bees (for the 3 groups) were taken from and returned to the same hive – the test groups were therefore not isolated from each other

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					and thus it is unknown whether a transfer of glyphosate and bacteria might have occurred among bees, - variations in gut bacteria are common in different hives, but also between different individuals, but there was no way to compare this information in the experiments, nor any information provided by the study authors regarding this, - the microbiome composition is influenced by age and since the age of the bees in the experiment was not reported, and it is possible that bees of different ages were used, it is not clear whether it is appropriate to compare between the groups in the experiment, - the exact amount of consumed sucrose syrup is not reported and the actual doses of glyphosate per bee per day therefore cannot be calculated, - the bees were not fed with pollen (a source of proteins and enzymes for nurse bees), which may have influenced the results since many microbes are dependent upon amino acids for survival. Taking into consideration the shortcomings listed above, the results of the hive experiment are not reliable and thus no conclusion can be drawn on glyphosate effect on gut microbiome size and absolute and relative abundances of the "core" species of the microbiome.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Colonization experiment "Approximately 100" newly emerged workers (NEWs), which are supposedly nearly free of gut bacteria, were simultaneously exposed to an inoculum consisting of their normal microbial community and to glyphosate (5 µl of sugar syrup with 1 mM glyphosate ~1.7 µg glyphosate/bee; bees were exposed twice within 2 days). Of these, 15 bees were sampled in order to determine gut microbiome. In a second colonization experiment the exposure levels differed, i.e. the bees were exposed to 0.1 mM glyphosate during 5 days. Since no more information was available, it is not possible to calculate the total dose the bees consumed and to quantitatively compare the effects between the tests. In this case, only 8 bees per test group were sampled for DNA and RNA extraction. In the first experiment, the average total bacterial abundance was slightly lower in glyphosate-treated bees, but this was not statistically significant. S. alvi was the most strongly affected member of the gut microbiota and its absolute and relative abundances were significantly lower in comparison with the control bees, while Lactobacillus Firm-4 increased in relative abundance only. The authors concluded that glyphosate exposure

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					during early development of the gut community can interfere with normal colonization by altering the abundance of beneficial bacterial species. In the second experiment, the authors also analyzed changes in bacterial abundance after glyphosate exposure by extracting both DNA and RNA from the guts of treated and control bees. A positive control group was included, in which bees were exposed to tylosin, an antibiotic used in beekeeping. This antibiotic treatment was expected to perturb the microbiota, but the decrease was significant only for RNA samples. Glyphosate exposure resulted in non-significant decreases in total bacteria for both DNA and RNA assays. Effects of glyphosate treatment on absolute abundance were specific to S. alvi, which was the only assayed species showing significant reductions in absolute abundance, observed for both DNA and RNA assays. The authors concluded that significant effects were observed only on absolute abundance of S. alvi DNA and RNA. Changes in gut microbiota composition following glyphosate exposure of age-controlled honey bees in the second colonization experiment. Boxplots of (A) 16S rDNA copies and (B) 16S rRNA

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					copies of total bacteria, Snodgrassella alvi, Lactobacillus Firm-4 and Firm-5 for control (C), 0.1 mM tylosin treated (T), and 0.1 mM glyphosate-treated (G) bees at post-treatment Day 0 (n = 8 for each group). Most of the shortcomings mentioned in the hive experiments are applicable here in the colonization experiment, e.g. (even smaller) sample size, unknown doses (per bee), lack of accounting for natural variations in gut microbiome among individuals. As a result, the colonization experiments are also considered unreliable. Infection experiments To determine whether glyphosate-induced perturbation of microbiota colonization affects host health, the susceptibility of glyphosate-treated bees to an opportunistic bacterial pathogen was measured in two experiments. NEWs were exposed to glyphosate during the stage of acquiring their normal microbial community. After 5d of treatment (first experiment: 0.1 mM glyphosate over 5 days; second experiment: 0.1 mM glyphosate over 5 d ~1.7 µg glyphosate/bee (assuming bees take on average 20 µl sucrose solution per day under captivity, the same likely applies for the first experiment, but it was not reported)), bees were challenged with Serratia marcescens

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					kz19, an opportunistic pathogen commonly detected at very low frequencies in the bee gut. For bees lacking gut microbiota, Serratia challenge resulted in increased mortality relative to that observed for bees with a conventional gut microbiota, regardless of glyphosate exposure. For bees with a conventional gut microbiota, glyphosate treatment resulted in increased mortality after Serratia challenge. In bees exposed to glyphosate, but not challenged with Serratia, survival rates were not significantly affected by glyphosate and much higher (they were actually the highest from all tested groups) than in the Serratia-challenged groups, demonstrating that a direct effect of glyphosate on bees is not the basis of the high mortality of glyphosate-exposed, pathogen-challenged bees. It was suggested by the authors, based upon the results above, that glyphosate reduces the protective effect of the gut microbiota against opportunistic pathogens and that S. alvi is the bacterial species most negatively affected by glyphosate exposure. The authors pointed out that S. alvi appears to give some immune protection, but not as fully as the whole gut microbiota.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The authors further hypothesize the reasons for the observed variation in sensitivity of certain strains of bacterial species toward glyphosate, which is outside of the scope of the present evaluation for ecological risk assessment. Only short conclusion on the topic is presented here: It was concluded that bee gut bacteria vary in glyphosate sensitivity at the species and strain levels and that S. alvi strains may vary in sensitivity to glyphosate in vivo. Thus, strain differences in glyphosate sensitivity may potentially contribute to the observed variation in the overall decrease in S. alvi abundance when bees with their native gut microbiota are exposed to glyphosate. It it is not clear how the dose of the injected pathogen relates to potentially realistic doses (i.e. to what levels of Serratia the bees are naturally exposed). The authors state that the pathogen is naturally present in the gut at low frequencies, so injecting the pathogen does not seem to reflect natural situation. It is also not clear whether the possible observed effects were a consequence of the infection itself, or a consequence of infection and exposure together to glyphosate (as bees have a higher energy demand when exposed to two stressors), or a consequence of alterations of the honey bee immune response as a result of unaccounted for factors.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Furthermore, the authors mention that the guts from 10 bees were pulled out, prepared, and were given to bees during 5 days until normal microflora was established. However, it is questionable what is the "normal" microflora, i.e. what is the baseline composition of the microflora. In addition, the bees were not fed with pollen (a source of proteins and important enzymes from nurse bees), which may influence the results as discussed above. Lastly, the doses were inferred by assuming that bees it 20 µl sucrose syrup per day, while in the EFSA GD on Bees (2013), the amount of the sugar bees consume is higher (32-128 and 34-50 mg/bee/day for foragers and nurses, respectively), and therefore the exact exposure dose reported is considered incorrect or at least uncertain. Overall, the colonization/infection experiments are considered not sufficiently reliable. Besides, although the study hypothesised that effects on the microbiome glyphosate may play a role in colony collapse, the positive control that was used for gut microbiome perturbation is an antibiotic commonly used in bee-keeping in some countries. Overall conclusion Several experiments were conducted in the study to show that exposure of

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					adult bees might impact the gut microbiome leading to a greater susceptibility to pathogen infections. On this aspect, the study is not directly relevant for the risk assessment but still relevant for the investigation of "other types of effects" not currently covered by the risk assessment scheme. It is considered by RMS as "additionnal data". However, based on a number of shortcomings in each experiment (e.g. very small sample sizes, unknown rearing and experimental conditions, influence of hive, individual and age on gut microbiome, lack of confirmation of the levels of actual glyphosate exposure per bee, bees diets had no source of amino acids, injection of a pathogen at unjustified levels, etc.), the study is considered unreliable.
Mottier A. et al.	2015	Effects of subchronic exposure to glyphosate in juvenile oysters (Crassostrea gigas): From molecular to individual levels.	Marine pollution bulletin (2015), Vol. 95, No. 2, pp. 665-77	Endpoints based on gene expressions are not considered in an ecotoxicology risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. Other parameters of direct relevance were also investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Mottier A. et al. 2015
Munoz L. M. H. *et al.*	2015	Toxicity assesment of two agrochemicals, Roundup Active and Cosmo-Flux411F,21 to colombian anuran tadpoles. Original title: Evaluación de la toxicidad de dos agroquímicos, Roundup® Activo y Cosmo-	Acta Biologica Colombiana (2015), Vol. 20, No. 2, pp. 153-161		The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable with restrictions. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		Flux®411F, en renacuajos de anuros colombianos.			
Murugan K. et al.	2014	Eudrilus eugeniae - a potent bioremediator in controlling herbicide and pesticide pollution.	International Journal of Pharmaceutical and Biological Archives (2014), Vol. 5, No. 5, pp. 67-74	Paper discusses the use of earthworm as bioremediation organisms to remove glyphosate from the soil. The endpoints presented are not relatable to an EU level risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Murussi C. R. et al.	2016	Exposure to different glyphosate formulations on the oxidative and histological status of Rhamdia quelen.	Fish physiology and biochemistry (2016), Vol. 42, No. 2, pp. 445-55	Findings cannot be related to an EU level ecotoxicology risk assessment	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Murussi C. R. et al. 2016
Mysore D. K. et al.	2013	Effect of metabolic inhibitors on growth and carotenoid production in Dunaliella bardawil.	Journal of food science and technology (2013), Vol. 50, No. 6, pp. 1130-6	Formulation tested contains tallow amine surfactant - not relevant to EU renewal.	The RMS does not agree with the applicant. The surfactant is not mentioned in the report. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Mysore D. K. et al. 2013
Nascentes R. F. et al.	2018	Low doses of glyphosate enhance growth, CO2 assimilation, stomatal conductance and transpiration in sugarcane and eucalyptus.	Pest management science (2018), Vol. 74, No. 5, pp. 1197-1205	This paper discusses hormetic responses of sugar cane and eucalyptus plants following exposure to low doses of glyphosate. The exposure situation and the presented endpoint data are not relevant to the EU level renewal of glyphosate	The RMS agrees with the applicant's reasoning and conclusion.
Nathan V. K. et al	2020	Pesticide application inhibit the microbial carbonic anhydrase-mediated carbon sequestration in a soil microcosm.	Environmental science and pollution research international (2020), Vol. 27, pp. 4468	5.4.1 case b) Relevant but supplementary information: Endpoints presented are not relevant to the direct effects assessment required for Annex I renewal. However, it does inform in other areas, e.g biodiversity / benefits of glyphosate use.	Data gap : Provide a study summary and assessment of relevance and reliability of the results related to glyphosate of the paper of Nathan V. K. et al, 2020, in particular for indirect effects and biodiversity assessment.
Navarro C. D. C. et al.	2014	Effects of the surfactant polyoxyethylene amine (POEA) on genotoxic,	Comparative biochemistry and physiology. Toxicology	Contains POEA, therefore not relevant to EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		biochemical and physiological parameters of the freshwater teleost Prochilodus lineatus.	& pharmacology (2014), Vol. 165, pp. 83-90		
Nevius B. A. et al.	2012	Surface-functionalization effects on uptake of fluorescent polystyrene nanoparticles by model biofilms.	Ecotoxicology (2012), Vol. 21, No. 8, pp. 2205	5.4.1 case b) Relevant but supplementary information: This paper discusses the results of an earthworm avoidance study which is not an endpoint type used in EU level risk assessment for Annex I renewal. Therefore it is considered to be supplementary. No effects were observed for glyphosate exposure.	The RMS does not agree with the applicant. However this study is not related to glyphosate. Not relevant.
Niemeyer J. C. et al.	2018	Do recommended doses of glyphosate-based herbicides affect soil invertebrates? Field and laboratory screening tests to risk assessment.	Chemosphere (2018), Vol. 198, pp. 154-160	The study is considered not relevant as it is conducted with Roundup Original. Despite the content being 360 g a.e./L, this product in Brazil is based on MON 78087, which contains MON 0818 which is a surfactant system containing POEA. This is not a relevant surfactant for the Annex I submission and therefore data generated using this formulation is not relevant to the EU Annex I renewal process from an ecototoxicology perspective.	The RMS does not agree with the applicant. The presence of POEA in Roudup Original is agreed but three other formulations were also tested in this study. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Niemeyer J. C. et al. 2012
Nocelli R. C. F. et al.	2019	Effects of herbicides on the survival of the brazilian native bee Melipona scutellaris latreille, 1811 (hymenoptera: apidae)	PLANTA DANINHA (2019), Vol. 37, pp. 1	End-points based on LT50 (time until 50% lethality, are not considered in an EU level bee risk assessment. Exposure scenario is not relevant to the risk assessment as bees were exposed for up to 45 days, being fed continuously and therefore not relatable to an exposure situation in the field.	The RMS does not agree with the applicant's reasoning and conclusion. LT50 is not directly relevant for the risk assessment but this study may still provide relevant information to be used in a Weight of evidence assessment. Besides the reports states they evaluated the impact in semi-field realistic conditions on temperature control and weight of colonies of M. scutellaris. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Nocelli R. C. F. et al. 2019
Nunez S. et al.	2015	In vitro effect of N-(phosphonomethyl)	Biocell (2015), Vol. 39, Suppl.	5.4.1 case b) Relevant but supplementary information: Endpoints based on the effects	The RMS does not agree with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		glycine agrochemicals on total heterotrophic bacteria and azotobacter chroococcum.	1. Abstract No.: A71.	of glyphosate on bacteria in soil are not considered in the EU level ecotox risk assessmen for Annex I renewal.	This study may provide relevant information for the indirect effects/biodiversity assessment. Only abstract was provided. Data gap: Provide the study together with a summary and a detailed assessment of reliability for the paper of Nunez S. et al. 2015
Nur Masirah M. Z. et al.	2013	Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: a microcosm experiment.	African Journal of Microbiology Research (2013), Vol. 7, No. 5, pp. 367-374	Non-EU monitoring study - not relatable to an EU level ecotoxicology risk assessment for Annex I renewal of glyphosate.	The RMS agrees with the applicant's reasoning and conclusion.
Nwani C. D. et al.	2013	Investigation on acute toxicity and behavioral changes in Tilapia zillii due to glyphosate-based herbicide, forceup.	JAPS, Journal of Animal and Plant Sciences (2013), Vol. 23, No. 3, pp. 888-892	The study was not conducted to GLP and a relevant guideline was not followed. The glyphosate formulation used in the study was Forceup and therefore the toxicity of the active substance to this fish species is unclear from this article. There was no rationale for the selection of exposure concentrations presented and no analytical verification of test concentrations was reported during the semi-static test procedure. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Nwani C. D. et al. 2013
Nwani C. D. et al.	2010	Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch).	International Aquatic Research (2010), Vol. 2, No. 2, pp. 105-111	Glyphosate products used to look at toxicity to Snakehead fish relevant to the Indian subcontinent. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU renewal	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Nwani C. D. et al. 2010

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Nweke C. O. et al.	2015	Prediction of phenolic compounds and formulated glyphosate toxicity in binary mixtures using Rhizobium species dehydrogenase activity.	Advances in Life Sciences (2015), Vol. 5, No. 2, pp. 27-38	Mixture study	The RMS agrees with the applicant's reasoning and conclusion.
Oliveira Souza C. et al.	2014	Exopolysaccharides and abiotic stress tolerance in bacterial isolates from "sabia" nodules.	Revista Caatinga (2014), Vol. 27, No. 4, pp. 240-245	Paper discusses a novel approach of assessing abiotic stress tolerance in bacterial isolates. Achieved dataset is not relatable to an EU level risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Olszyk D. et al.	2010	Phytotoxicity assay for seed production using Brassica rapa L.	Integrated environmental assessment and management (2010), Vol. 6, No. 4, pp. 725-34	Development of an assay to look at impact of glyphosate (and other pesticides) on the seed production of plant species with a short life cycle. End-points cannot be used in the regulatory risk assessment of glyphosate.	The RMS does not agree with the applicant's reasoning and conclusion. Despite not directly relevant for the risk assessment this study may still provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Olszyk D. et al. 2010
Olszyk D. et al.	2010	Potato (Solanum tuberosum) greenhouse tuber production as an assay for asexual reproduction effects from herbicides.	Environmental toxicology and chemistry (2010), Vol. 29, No. 1, pp. 111-21	Study to look at effect of glyphosate product (and other chemistry) on potato plants asexual reproduction to develop an assay. EC25 values generated for glyphosate and effect on fresh weight on potato tuber and shoot weight. Not relevant to the regulatory risk assessment of glyphosate renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Orsted M. et al.	2015	A fluorescence-based hydrolytic enzyme activity assay for quantifying toxic effects of Roundup (R) to Daphnia magna.	Environmental Toxicology and Chemistry (2015), Vol. 34, No. 8, pp. 1841-1850	Describes a novel fluorescence technique that is not relevant to EU level ecotoxicology risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Orun I. et al.	2013	Effects of acute and chronic exposure to glyphosate on common carp (Cyprinus carpio L.) hematological parameters: the beneficial effect of propolis.	Fresenius Environmental Bulletin (2013), Vol. 22, No. 9, pp. 2504-2509	The article does not report results, which can be used for ecotoxicology risk assessment for Annex I renewal purposes. Contains cellular and molecular findings	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Owagboriaye F. et al.	2020	Biochemical response and vermiremediation assessment of three earthworm species (Alma millsoni, Eudrilus eugeniae and Libyodrilus violaceus) in soil contaminated with a glyphosate-based herbicide.	Ecological Indicators (2020), Vol. 108, pp. 105678	Endpoints based on effects on enzymes levels in earthworms are not used in the EU level ecotoxicology risk assessment for renewal under Annex I. Concerning exposure, based on 83.2g a.i./m^2 equivalent, the corresponding application rate per hectare is 832,000 g/ha, equal to 832 kg/ha. This rate far exceeds proposed EU application rate max of 2.16 kg/ha. Therefore findings are difficult to relate to an EU exposure scenario. The product used was also not the representative formulation proposed in the Annex I dossier.	Overall, in view of the exposure, the RMS accepted to exclude the paper..
Pala A.	2019	The effect of a glyphosate-based herbicide on acetylcholinesterase (AChE) activity, oxidative stress, and antioxidant status in freshwater amphipod: Gammarus pulex (Crustacean).	Environmental science and pollution research international (2019), Vol. 26, No. 36, pp. 36869-36877	Roundup was used which contains POEA. This is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant. The presence of POEA was not stated in the report. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Pala A. 2019
Paganelli A. *et al.*	2010	Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling.	Chemical research in toxicology (2010), Vol. 23, No. 10, pp. 1586-1595	5.4.1 case b) Relevant but supplementary information. Study was conducted in Argentina. Very high concentrations were tested and an unrealistic route of exposure was examined (glyphosate was injected into embryos). In addition, the tested formulation is not the representative formulation for the glyphosate EU renewal.	The RMS agrees that injection of glyphosate into embryos is not an exposure route representative for field conditions. The study has 2 major limitations, namely lack of analytical verification and suboptimal design of the control group (no real 'procedural' control). The RMS concludes that the study is not relevant and not reliable.
Panda N. et al.	2016	Herbicides impact on Fe and Mn reduction and dehydrogenase activity in an agricultural soil.	Journal of Crop and Weed (2016), Vol. 12, No. 3, pp. 142-149	Comparitive effects on Fe and Mn transformation and dehydrogenase activity in soils are not endpoints used in the EU level ecotoxicology risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Panettieri M. et al	2013	Glyphosate effect on soil biochemical properties under conservation tillage	Soil & tillage research (2013), Vol. 133, pp. 16	5.4.1 case b) Relevant but supplementary information: The paper describes different tillage techniques following use of glyphosate and the impact on soil properties. Not relateable directly	Data gap: Provide a summary and assessment of relevance and reliability for use in biodiversity assessment of the paper of Panettieri M. et al. 2013.

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				to risk assessment for renewal but may be useful in the biodiversity and benefits discussions.	
Panetto O. S. et al.	2019	The effects of Roundup® in embryo development and energy metabolism of the zebrafish (Danio rerio)	Comparative biochemistry and physiology (2019), Vol. 222, pp. 74	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B) The test concentrations in the test system were not analytically verified and therefore, exposure concentrations cannot be confirmed. The study is considered unreliable.	The study authors indicates that Roundup Original®, was used in this study, which presents in addition to glyphosate, surfactants substances that increase the effect power of the herbicide (reference is made to Sánchez et al., 2017). Sanchez et al 2017 states that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." This indicates the presence of POEA in the formulation tested. POEA is not authorized in plant protection products containing glyphosate (European Commission, August 2016). Due to presence of POEA, the study is considered not relevant by RMS.
Panwen M. et al.	2013	Acute toxicity of pesticides glyphosate and paraquat on river snails	Siliao Yanjiu (2013) No. 11, pp. 44	5.4.1 case b) Relevant but supplementary information: The material and methods sections lack important information. The test organisms were not specified. Detailed information on preparation and application of test solution is missing. The tested concentrations and the exposure time were not reported in the material	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				and methods. The test item is not specified. It is only stated that it contains 10 % active ingredient, but other ingredients are unknown. No control results are available. Furthermore, it is unclear whether the reported endpoints refer to the active substance or to the product. No analytical verification of test concentrations were performed. The study is considered unreliable.	
Pasini R. A. et al.	2018	Comparative selectivity of herbicides used in wheat crop on the predators Chrysoperla externa and Eriopis connexa	Planta Daninha (2018), Vol. 36,pp. E018179968	5.4.1 case b) Relevant but supplementary information: In the material and methods section important information is missing. The test items were not adequately specified regarding the content of the active ingredient. It is unclear whether the given active ingredient concentration in the spray solution corresponds to the content of the active ingredient in the formulation. The test did not follow a specific test guideline, although the culturing of the insects was conducted according to recognised approaches. There were no validity criteria established and the performance of the assays was not assessed using a positive control substance. An endpoint that could be used in an ecotoxicology risk assessment was not established.	Glyphosate was considered as innocuous to the stages of larva, egg, and pupa of C. externa and E. connexa. However RMS agrees with the applicant's conclusion. No reliable endpoint can be derived.
Patkowski M. et al.	2016	Response of soil phosphatases to glyphosate and its formulations - Roundup (laboratory conditions).	Plant, Soil and Environment (2016), Vol. 62, No. 6, pp. 286-292	Technical data cannot be related to an EU level Annex I ecotoxicology risk assessment. Formulations used contain POEA.	The RMS does not agree with the applicant. The presence of POEA was stated for only one of the two formulations tested. However it is agreed that the data from this study are not relatable to the risk assessment. Study not relevant.
Peel M. D. et al.	2013	Natural Glyphosate Tolerance in Sainfoin (Onybrychis viciifolia).	Crop Science (2013), Vol. 53, No. 5, pp. 2275-2282	Paper discusses tolerance to glyphosate a comparison with two plants types. Endpoints specific to EU level ecotoxicology risk assessment are not presented.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Pereira J. L. et al.	2018	Effects of glyphosate on the non- target leaf beetle Cerotoma arcuata (Coleoptera: Chrysomelidae) in field and laboratory conditions.	Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes (2018), Vol. 53, No. 7, pp. 447-453	The work was based on populations associated with applications made to glyphosate resistant soybeans. Over the top applications to traited crops are not relevant to the Annex I GAP table. The formulation used was Roundup Original, which contains POEA and is not included in the representative formulation (MON 52276), therefore findings of the study based on a formulation containing POEA are not considered relevant to the EU level RA. for Annex I purposes.	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roudup Original, the study is considered not relevant by RMS.
Pereira P. C. et al.	2019	Acute Toxicity of Herbicides and Sensibility of Aquatic Plant Wolffia brasiliensis as a Bioindicator Organism	Planta Daninha (2019), Vol. 37, pp. e019201636	5.4.1 case b) Relevant but supplementary information: This paper describes a non-standard aquatic plant ecotoxicity test for a non-EU native species, and is therefore difficult to relate to an EU level ecotox risk assessment. The formulation used is specific to aquatic applications that are not on the proposed GAP for the renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Persch T. S. P. et al.	2018	Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup(®), Primoleo(®) and Facet(®)).	Environmental toxicology and pharmacology (2018), Vol. 58, pp. 170-179	Relates to snails being exposure to a formulation of glyphosate that is not the representative formulation at Annex I for the EU.	The RMS does not agree with the applicant. Glyphosate exposure caused an imbalance in the oxidative status. No quantitative link can be made between these effects and potential effect at population level. These data are not relatable to the risk assessment.
Pfleeger T. et al.	2014	Effects of single and multiple applications of glyphosate or aminopyralid on simple constructed plant communities.	Environmental toxicology and chemistry (2014), Vol. 33, No. 10, pp. 2368-78	This paper looks at response of plant communities following multiple application scenarios across multiple years. Endpoints in terms of plant volume are not relatable to and EU level Ecotoxicology risk assessment for Annex I renewal.	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup

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					Original formulation. The MON 0818 (a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roundup Original, the study is considered not relevant by RMS.
Pfleeger T. et al.	2010	Comparing effects of low levels of herbicides on greenhouse- and field- grown potatoes (Solanum tuberosum L.), soybeans (Glycine max L.), and peas (Pisum sativum L.)	Environmental Toxicology and Chemistry (2010), Vol. 30, No. 2, pp. 455-468	Study to compare effects of glyphosate on greenhouse and field grown potatoes, soybean and peas to determine if greenhouse studies are protective of field conditions. Conducted by US EPA. EC25 values generated but as the paper is comparing effect in and outside the greenhouse, the data does not contribute to the regulatory risk assessment of the glyphosate renewal.	The RMS does not agree with the applicant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Pfleeger T. et al. 2010
Piotrowicz- Cieslak A. I. et al.	2010	Different Glyphosate Phytotoxicity of Seeds and Seedlings of Selected Plant Species.	Polish Journal of Environmental Studies (2010), Vol. 19, No. 1, pp. 123	5.4.1 case b) Relevant but supplementary information: Study to compare the effect of glyphosate on plant growth parameters of 6 plant species.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Piotrowicz- Cieslak A. I. et al. 2010
Pizarro H. et al.	2016	Impact of multiple anthropogenic stressors on freshwater: how do glyphosate and the invasive mussel Limnoperna fortunei affect microbial communities and water quality?.	Ecotoxicology (2016), Vol. 25, No. 1, pp. 56-68	The paper looks at the synergistic effects of glyphosate and freshwater mussels on eutrophication in surface waters. Not relatable to an EU level ecotoxicology risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Pochron S. et al.	2019	Temperature and body mass drive earthworm (Eisenia fetida) sensitivity to a popular glyphosate-based herbicide	Applied soil ecology (2019), Vol. 139, pp. 32-39	Formulation used is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Pochron S. et al. 2019

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Pochron S. et al.	2020	Glyphosate but not Roundup® harms earthworms (Eisenia fetida).	Chemosphere (2020), Vol. 241, pp. 125017	5.4.1 case b) Relevant but supplementary information: The study was not conducted to GLP. The test design does not correspond to a current test guideline for earthworms focusing on reproduction parameters and there is no endpoint for risk assessment. Only a single dose level was used in the test, which is equivalent to 19.7 kg/ha; substantially higher than the maximum proposed application rate of glyphosate for the renewal. There was no analytical confirmation of levels tested, so exposure cannot be confirmed.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant data for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Pochron S. et al. 2020
Polyakova N. N. et al.	2018	Effect of herbicides application on the soil biological activity in the tree nursery	Agrokhimiya (2018), Vol. 12, pp. 35-41	The paper describes the use of buried linen to establish the activity of microorganisms in the soil during a 2 year monitoring period in a tree nursery. The observations cannot be related to an EU level risk assessment as they are based on visual inspection / qualitative assessment of the amount of apparent breakdown of the linen.	The RMS agrees with the applicant's reasoning and conclusion.
Prevot-D'Alvise N. et al.	2013	Acute toxicity of a commercial glyphosate formulation on European sea bass juveniles (Dicentrarchus labrax L.): gene expressions of heme oxygenase-1 (ho-1), acetylcholinesterase (AChE) and aromatases (cyp19a and cyp19b).	Cellular and molecular biology (2013), Vol. 59 Suppl, pp. OL1906	5.4.1 case b) Relevant but supplementary information: Test item was appropriately identified as being linked to the representative formulation. Test design does not however follow a recognised approach, uneven sample sizes and large fish were exposed. The rationale behind test concentration selection was not clear and dose preparation was unclear as exposure rates could not be confirmed. Effects of acetone on fish were not discussed. Endpoints anyway demonstrate low toxicity compared to existing list of endpoints.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant data for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Prevot-D'Alvise N. et al. 2013
Puertolas L. et al.	2010	Evaluation of side-effects of glyphosate mediated control of giant reed (Arundo donax) on the structure and function of a nearby Mediterranean river ecosystem.	Environmental research (2010), Vol. 110, No. 6, pp. 556	5.4.1 case b) Relevant but supplementary information	The effect of the herbicide Herbolex (mixture of glyphosate isopropylamine salts and surfactant compounds) on the structure and function of a nearby river ecosystem after application of glyphosate in the riparian vegetation was evaluated. In

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					situ bioassays with transplanted Daphnia magna, field collected caddis fly (Hydropsyche exocellata) and benthic macroinvertebrate structure and function were investigated. The herbicide was applied at 2.1 kg glyphosate/ha in an area of 0.5 ha of riparian forest. Transplants with Daphnia magna were deployed at the day of application and 12 days afterwards, whereas Hydropsyche exocellata samples were collected at the day of application and 3 days afterwards. Concentration of glyphosate and the metabolite AMPA was analysed in the river water samples collected from the studied sites at the day of application and two, three and 12 days afterwards. No exact biological data regarding the macroinvertebrate abundance is reported. No effect was observed but it may be explained by a less diverse community dominated by tolerant species (according to study authors). Significant specific toxic effects on transplanted D. magna and field collected H. exocellata were observed. Measured glyphosate concentrations ranging between 20 and 137 µg/l in water affected D magna feeding rates and the activity of biotransformation (GST, CbE), antioxidant (GPX), metabolic (LDH) and/or

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					anticholinergic enzymes of the two studied invertebrate species. In each in situ D. magna deployments, a lab control treatment with animals maintained in the lab and never exposed to the field was included as a surrogate control. The authors suggested interactive combined effects of naturally occurring factors and the herbicide application. Indeed the studied sites were characterized by difference in organic pollution (L2 received the sewage of a nearby small industrial park) and salinization (G was highly impacted by an excess of Cl and SO4). An excess of ammonium coming from water treatment plant, salts and changes in oxygen levels affected the studied behavioral and biochemical responses. Overall, due to poor water quality, RMS would not consider the results reliable. RMS questions the comparison using lab controls (no in situ control was available). Effects may also be due to any other parameters related to the study site. The study is less relevant (due to different formulation tested) and not reliable.
de Oliveira Procopio S. et al.	2014	Toxicity of herbicides used in the sugarcane crop to diazotrophic bacterium Herbaspirillum seropedicae.	Semina: Ciencias Agrarias (2014), Vol. 35, No. 5, pp. 2383-2398	Paper discusses the impact of glyphosate on cell densities of soil bacteria tested in liquid culture and using novel assay approaches. The endpoints	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				cannot be related to an EU level ecotoxicology risk assessment for renewal.	
Qin Y. et al.	2017	Toxic effects of glyphosate on diploid and triploid fin cell lines from Misgurnus anguillicaudatus.	Chemosphere (2017), Vol. 180, pp. 356-364	Cellular level endpoints cannot be related to the ecotoxicology Annex I renewal risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Rahnama R. et al.	2018	Acute toxicity of herbicides on the survival of adult shrimp, Artemia Franciscana	Iranian Journal of Toxicology (2018), Vol. 12, No. 6, pp. 45	5.4.1 case b) Relevant but supplementary information: Important information is missing in the material and methods section. The preparation and application of the test solutions was not reported. The test item is not adequately specified. The given purity of 41 % indicates that a product was tested. However, it is not clear whether the test concentrations refer to the product or to the active substance. In addition, the biological results of the test were not sufficiently stated. The endpoint data presented in the paper is difficult to understand. Table 3 in the article indicates a 48 hour LC50 of 17.483 mg/L, whilst in Figure 2, the 48 hour LC50 is 38.897 mg/L. Therefore, the reliability of the data presented in the article is questionable. In addition, it is unclear whether the animals were fed during the assay. Figure 3 appears to show artemia with egg bags and highlights the contents of the rudimentary artemia gut as being those exposed to herbicides. This observation is not supported by any information presented in the paper. No mortality data for the test concentrations nor for the controls is presented to evaluate the results. Assessment of the statistical power of the assay is not possible. Furthermore, there was no analytical verification of test concentrations reported, there is no guideline stated and it is non GLP. Multiple doses were tested, but a positive control group was not included, so the performance / robustness of the test system	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				cannot be confirmed. The study is considered unreliable.	
Rainio M. J. et al.	2019	Effects of a glyphosate-based herbicide on survival and oxidative status of a non-target herbivore, the Colorado potato beetle (Leptinotarsa decemlineata)	Comparative biochemistry and physiology. Toxicology & pharmacology (2019), Vol. 215, pp. 47	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B	The direct toxic effects of Roundup® Bio, containing 360 g/L glyphosate, on survival of Colorado potato beetles (Leptinotarsa decemlineata) were investigated. Beetles originating from different continents from different populations were used to compare of the susceptibility to glyphosate exposure. Newly hatched larvae (3–6 days old) were tested at the concentrations of 100 and 1.5 % Roundup® Bio (360 and 5.4 g glyphosate isopropylamine salt/L). Larvae were treated to Roundup by pipetting a small drop (3 µL) on top of the larvae. Observations were made after 2, 24, 48, 72 and 96 hours. Only highest dose induced effects. The dose rate tested is considered unrealistically high as mentioned by study author. The study is not relevant. Reliability was not assessed.
Ranganathaswamy M. et al.	2012	Evaluation of toxicity of agrochemicals on Trichoderma isolates in vitro.	Journal of Biological Control (2012), Vol. 26, No. 4, pp. 391	5.4.1 case b) Relevant but supplementary information: The form of glyphosate used in the experiments cannot be confirmed. Fungal growth inhibition is not part of the specific ecotox risk assessment for the renewal.	The RMS does not agree with the applicant's reasoning and conclusion. Effects on fungal growth are considered a relevant information for the indirect effects/biodiversity assessment. However no relevant endpoint can be derived from this study to be used in the risk assessement.
Reddy S. B. et al.	2018	Disturbances in reproduction and expression of steroidogenic enzymes	Toxicology Research and Application (2018), Vol. 2, pp. 239784731880527 6/1	Findings cannot be related to an EU level ecotoxicology risk assessment, as the methods used are not recognised at the EU level.	Effects of the individual active ingredients of the herbicide Roundup (glyphosate and diquat dibromide

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		in aquatic invertebrates exposed to components of the herbicide Roundup			[DD]) were investigated in the aquatic snail Lymnaea palustris. Glyphosate was tested at 3.5 mg/L: -Fecundity in glyphosate treated snails is comparable to or exceeds control levels. -Levels of steroid acute regulatory (StAR) protein in whole snails decrease with treatment with Roundup, glyphosate, or DD. -StAR in organs where steroid biosynthesis occurs (ovotestis, brain, kidney) reduced following chronic exposure to Roundup, glyphosate, or DD ($p < 0.01$). -Testosterone levels decrease in DD-treated groups ($p < 0.05$); a trend of lower testosterone is also observed in glyphosate-treated groups ($p > 0.05$). -Estradiol concentration is greater than or equal to control levels in glyphosate but decreased in DD ($p < 0.05$). -Because of its role in the conversion of testosterone to estradiol, abundance of aromatase was monitored. A reduction ($p < 0.05$) was observed in DD-treated snails (consistent with the drop in fecundity and estradiol levels) and a comparable level to control in glyphosate-treated snails (consistent with their high fecundity and estradiol levels). Overall: Results corresponding to both glyphosate and diquat dibromide are reported in the summary.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					The study supports that Roundup and its constituents (glyphosate and DD) are endocrine disruptors. RMS notes that the concentration tested (3.5 mg/L) is higher than the concentrations expected in real conditions of use. The report also states that a "low" level of spontaneous mortality occurred. It is not known to what extend this letality has affected the results (no biological data presented for mortality). No MTC could be determined (no mortality data, only one tested concentration). No analytical verification was conducted. The results from this study (for glyphosate) are relevant for the assessment of potential for endocrine disruption. The study is relevant but unreliable (as it cannot be excluded that effects are due to systemic toxicity).
Reis L. A. C. et al.	2018	Leaf morphoanatomy and biochemical variation on coffee cultivars under drift simulation of glyphosate.	Planta Daninha (2018), Vol. 36, pp. E018143560	Findings not relatable to an EU level ecotoxicology risk assessment for product renewal in the EU.	The RMS agrees with the applicant's reasoning and conclusion.
Reno U. et al.	2016	Efectos subletales de cuatro formulaciones de glifosato Sobre daphnia magna y ceriodaphnia dubia (crust acea, cladocera)	Natura Neotropicalis (2016), Vol. 47, No. 1, pp. 7	5.4.1 case b) Relevant but supplementary information: The aim of the study was to compare the chronic toxicity of four different commercially available glyphosate products to Daphnia magna and Ceriodaphnia dubia. The study was not conducted according to GLP and the study design lacks some details compared with relevant guidelines. The test concentrations are based on nominal and no analytical verification of test item concentrations were	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				conducted (only analysis of stock solutions using an unspecific detector). Although the details of the statistical analyses are reported, the study report only describes where significant differences were found. No detailed results including standard deviations of the investigated parameters are provided. As the study is based on different glyphosate products, the toxicity of glyphosate active substance alone is unknown and therefore endpoints generated from this study are not quantifiable and deliver only supplementary information.	
Reno U. et al.	2018	Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species.	Ecotoxicology (2018), Vol. 27, No. 7, pp. 784-793	Formulation used is not the representative formulation for the Annex I renewal.	Acute effects of 4 glyphosate formulations were investigated on two freshwater cladoceran species (Daphnia magna and Ceriodaphnia dubia). This study aims to predict the population dynamics and the potential for recovery of exposed organisms (using population model). Eskoba®, Panzer Gold®, Roundup Ultramax® and Sulfosato Touchdown® were used in both cladoceran species through acute tests and 15-day recovery tests in order to estimate the population dynamics of microcrustaceans. The study reports LC50 values for Daphnia magna for the 4 formulations. They all indicate far lower LC50 than those available for the active substance alone. Ceriodaphnia dubia seems more sensitive than Daphnia magna. In view of the formulation description, the use of the results to assess the toxicity of glyphosate as formulated in MON52276 is questionable. Only

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					LC50 values are presented, no biological data is available. Analytical verification may not have been conducted. The reliability of the model predictions was not assessed by RMS. Therefore RMS considers that this study is less relevant but supplementary (due to the different formulation tested) and not reliable.
Reno U. et al.	2016	Water polluted with glyphosate formulations: effectiveness of a decontamination process using *Chlorella vulgaris* growing as bioindicator	Journal of applied phycology (2016), Vol. 28, No. 4, pp. 2279-2286	Study conducted using Roundup ultramax (AKA Mon 78784) that contains surfactants that are different to those used in MON 52276. Therefore findings cannot be related to the ecotoxicology assessment for Annex I renewal for MON 52276	Publivcation related to effectiveness of decontamination process not usable for ecotox risk assessment. The RMS agrees with the applicant's reasoning and conclusion.
Rezende-Silva S. L. et al.	2019	Pouteria torta is a remarkable native plant for biomonitoring the glyphosate effects on Cerrado vegetation	Ecological indicators (2019), Vol. 102, pp. 497- 506	Formulation not the representative formulation for the Annex I renewal. Enzymatic endpoints are not relevant to an EU level risk assessment	Roundup Transorb was used. Surfactants were not stated in this study. It is very likely that the tested formulation included the surfactant polyethoxylated tallow amine (POEA) as in an other study Rocha T. L. et al., 2015, (also conducted in Brazil) "Transorb" was used. In this study it was clearly stated that Transorb contains POEA. The study is considered not relevant by RMS.
Richard S. et al.	2014	Effect of a glyphosate-based herbicide on gene expressions of the cytokines interleukin-1β and interleukin-10 and of heme oxygenase-1 in European sea bass, Dicentrarchus labrax L.	Bulletin of environmental contamination and toxicology (2014), Vol. 92, No. 3, pp. 294-9	End-points based on gene expression are not considered in the EU level Annex I ecotoxicology risk assessment for renewal.	The RMS agrees with the applicant's reasoning and conclusion particularly as effects on gene could not be related to effects on individuals or population.
Rocha T. L. et al.	2015	Proteomic and histopathological response in the gills of Poecilia reticulata exposed to glyphosate-based herbicide.	Environmental toxicology and pharmacology (2015), Vol. 40, No. 1, pp. 175-86	Observed findings relate to a formulation that is not the representative formulation for the Annex I renewal in the EU.	Roundup Transorb was used. The study authors indicated the the tested formulation was more toxic to fish than pure glyphosate and that higher toxicity was linked to the toxic

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					effects of surfactant POEA or synergistic effect between the different components of the formulation. In this study, it is clearly stated by the study authors that the tested formulation included the surfactant polyethoxylated tallow amine (POEA). Since August 2016, POEA is not authorized in plant protection products containing glyphosate (European Commission). Therefore it is not possible to discriminate between glyphosate and POEA. The study is considered not relevant by RMS.
Rodriguez A. M. et al.	2018	Glyphosate Alters Aboveground Net Primary Production, Soil Organic Carbon and Nutrients in Pampean Grasslands (Argentina)	Rangeland ecology & management (2018), Vol. 71, No. 1, pp 119-125	This paper describes multi season monitoring of different habitats in Argentina, the findings of which cannot be related to an EU level risk assessment for Annex I renewal from an ecotoxicology perspective.	The RMS agrees with the applicant's reasoning and conclusion.
Romano-Armada N. et al.	2019	Construction of a combined soil quality indicator to assess the effect of glyphosate application.	The Science of the total environment (2019), Vol. 682, pp. 639-649	Paper describes a new approach to establishing the quality of farmland soils by assessing multiple physical, chemical and biological quality factors of soils and attempting to classify these as being of high or low quality based on a known history of glyphosate or no glyphosate application. To this end, the paper does not describe endpoint data that can be related to an EU level Annex I submission.	The RMS does not agree with the applicant's reasoning and conclusion. This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Romano-Armada N. et al. 2019
Rondon Neto R. M. et al.	2011	Phytotoxicity of Aspidosperma desmanthum under glyphosate drifting. Fitotoxidade de peroba-mica (Aspidosperma	Revista Brasileira de Herbicidas (2011), Vol. 10, No. 2, 103 p	The study looks at the toxicity of glyphosate on seedlings of a tree species that is native to South America, Mexico and West Indies. The test substance was Gliz 480 SL (IPA salt). Due to the test materials not being the representative	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		desmanthum) submetidas a deriva de glyphosate.		formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Rondon Neto R. M. et al. 2011
Roongruangchai J. *et al.*	2018	The teratogenic effects of glyphosate based herbicide (GBH) on the development of chick embryos	Siriraj Medical Journal (2018), Vol. 70, No. 5, pp. 419 28	The exact composition of the Roundup formulation tested is not stated in the paper (a.i. content, source, surfactant system / co-formulants). Furthermore, in the absence of a concurrent control for each of the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or to one of the other components	The exposure method (injection into embryos) is not an environmentally-realistic exposure pathway and hence this study is not considered relevant for the risk assessment. Moreover, due to high embryo mortality, it is likely that the developmental effects were a result of systemic toxicity, and hence the results are not relevant for assessment of ED properties.
Roy N. M. et al.	2016	Glyphosate induces neurotoxicity in zebrafish.	Environmental toxicology and pharmacology (2016), Vol. 42, pp. 45-54	Endpoint not used in EU level ecotoxicology risk assessment	The RMS does not agree with the applicant's reasoning and conclusion. Despite not directly relevant for the risk assessment this study may still provide relevant information to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Roy N. M. et al. 2016
Ruiz-Toledo J. et al.	2014	Effect of the concentration of glyphosate present in body waters near transgenic soybean fields on the honeybee Apis mellifera, and the stingless bee Tetragonisca angustula. Efecto de la concentracion de glifosato presente en cuerpos de agua cercanos a ca	Acta Zoologica Mexicana (2014), Vol. 30, No. 2, pp. 408-413	Non-EU monitoring study. Extrapolation to EU difficult.	The RMS agrees with the applicant's reasoning and conclusion.
Rzymski P. et al.	2013	The effect of glyphosate-based herbicide on aquatic organisms - a case study.	Limnological Review (2013), Vol. 13, No. 4, pp. 215	5.4.1 case b) Relevant but supplementary information: This paper describes the impact of an episodic pollution event on Lake Lednica in Poland, describing monitoring results for	This paper describes the impact of an episodic pollution event on Lake Lednica in Poland, describing monitoring results for glyphosate

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				glyphosate detects in lake water and the apparent impact on various aquatic taxa groups, in comparison to a control station site established at another location. These data cannot be related to a specific use or application rate and therefore are not relatable to the EU ecotoxicological regulatory risk assessment for the glyphosate EU renewal.	detects in lake water and the apparent impact on various aquatic taxa groups, in comparison to a control station site established at another location. Glyphosate concentrations were measured. Studies of benthic macroinvertebrates revealed no significant differences in taxa number between event (13 taxa) and control (14 taxa) sites although differences in abundance of particular taxa were observed. RMS notes that differences between sites were likely even before the pollution event. No data was available to confirm that abundances in both sites were similar (before the event). Relevant but not reliable for regulatory risk assessment.
Saba R. M. et al.	2018	Toxicological and biochemical investigation of certain herbicides on Culex pipiens L. (Diptera: Culicidae) mosquitoes under laboratory conditions	Advances in Natural and Applied Sciences (2018), Vol. 12, No. 2, pp. 6-12	Non-representative formulation (Herbazd 48% EC) was tested. Test organisms were field collected with no knowledge of prior exposure to chemicals. Important information is missing in the material and methods section. The preparation and application of the test solutions as well as the tested concentration range were not reported. The test item is not adequately specified. Although the herbicide formulation is given with a purity of 48 %, it is not clear whether the test concentrations refer to the product or to the active substance. Moreover, the active ingredient is given as glyphosate isopropylamine which should be formulated as a salt resulting in test concentrations as acid equivalents. In addition, the biological results of the test were not sufficiently stated. No mortality data neither for the test concentrations nor for the controls was given to evaluate the results. Furthermore, there	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				was no analytical verification of test concentrations reported, there is no study guideline and the study is non-GLP and thus the reliability of the study and its relatability to an EU level ecotoxicology risk assessment is questionable.	
Sadeghi A. et al.	2014	Investigation of LC50, NOEC and LOEC of glyphosate, deltamethrin and pretilachlor in guppies (Poecilia reticulata)	Iranian Journal of Toxicology (2014), Vol. 8, No. 26, pp. 1124	5.4.1 case b) Relevant but supplementary information: Study was considered to be conducted according to a recognised guideline via the cited reference in the paper, but the test system specifics cannot be confirmed. For example, there are validity criteria stated but water qualities / environmental conditions are not presented, so the suitability of the test system cannot be confirmed. Additionally, there was no analytical verification of the exposure concentrations, so exposure cannot be confirmed. The source and age / size of the fish are not presented in the paper, so the appropriateness of the test system cannot be confirmed. Additionally, the size of the aquariums used is stated (120 L) but the volume of test or control medium in these vessels is not stated, therefore fish loading rates cannot be determined. The test substance is identified as a 'commercial 41% glyphosate' – no other information are presented so effects cannot clearly be related to the active substance glyphosate, and the relevance of the test item used to the EU renewal of MON 52276 cannot be confirmed. The study is considered unreliable.	The study is considered as less relevant but supplementary based on RMS criteria. RMS agrees with justifications provided. The study is not reliable.
Salgado T. P. et al.	2011	Initial symptoms of Eucalyptus intoxication by glyphosate rates applied on the stem or leaves. Sintomas da intoxicacao inicial de Eucalyptus proporcionados por subdoses de glyphosate	Planta Daninha (2011), Vol. 29, No. 4, pp. 913	5.4.1 case b) Relevant but supplementary information: Effects on eucalyptus seedlings after application of glyphosate (Roundup Original, 360 g a.e./L). Spraying the aerial part of the plants (trials 3 and 4). Plant BBCH stage unclear (hight at start of application: 40/ 69 cm). No biological results for control or any test	The presence of POEA is not mentioned in the study. However in an other study (Sanchez et al 2017) it was stated that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		aplicadas no caule ou nas folhas.		concentration reported in tables. Therefore the results cannot be reproduced. No results in values which can be used for the risk assessment.	(a code of Monsanto for designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." Due to presence of POEA in Roundup Original, the study is considered not relevant by RMS.
Salbego J. et al.	2014	Glyphosate on digestive enzymes activity in piava (Leporinus obtusidens).	Ciencia Rural (2014), Vol. 44, No. 9, pp. 1603-1607	Study provides information on cellular/molecular level and is no ecotoxicological relevant study	The RMS agrees with the applicant's reasoning and conclusion given that no link with effect on individual/population is possible based on this paper.
Salman J. M. et al.	2016	Effect of pesticide glyphosate on some biochemical features in cyanophyta algae oscillatoria limnetica.	International Journal of PharmTech Research (2016), Vol. 9, No. 8, pp. 355-365	Cellular and molecular level endpoints discussed that are not relevant to EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion given that no link with effect on individual/population is possible based on this paper.
Salvio C. et al.	2016	Survival, Reproduction, Avoidance Behavior and Oxidative Stress Biomarkers in the Earthworm Octolasion cyaneum Exposed to Glyphosate.	Bulletin of environmental contamination and toxicology (2016), Vol. 96, No. 3, pp. 314-9	The earthworm were collected in field in Argentina and acclimatized for 2 weeks. Therefore, the organisms may have had exposure to other chemicals in the field. No information on the field history regarding application of pesticides is known. Analytical measurements of glyphosate were carried out in the short-term bioassay samples. While the test organisms, test design and procedure are well described and all information for the evaluation of the study is given, no endpoint considered relevant for use in risk assessment was determined. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The study is considered less relevant but supplementary based on RMS criteria. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Salvio C. et al. 2016
Samal S. et al.	2019	Setal anomalies in the tropical earthworms Drawida willsi and Lampito mauritii exposed to elevated concentrations of	Environmental technology & innovation (2019), Vol. 15, pp. 100391	Paper describes a electron microscopic approach for establishing effects of pesticides on setal in worms. The findings are not relatable to an EU level ecotoxicological risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		certain agrochemicals: An electron micrographic and molecular docking approach			
Samal S. et al.	2019	Evaluating the effect of monocrotophos and glyphosate on microbial population and certain important exoenzyme activities in soil.	Journal of Environmental Biology (2019), Vol. 40, No. 2, pp. 226	5.4.1 case b) Relevant but supplementary information: Dosing information / purity of both active substances cannot be confirmed. Study not conducted according to a recognised guideline. Presented endpoints not relateable to an EU level risk assessment based on lack of soil characterisation.	The RMS does not agree with the applicant's reasoning and conclusion. This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Samal S. et al. 2019
Samanta P. et al.	2014	Biochemical effects of glyphosate based herbicide, Excel Mera 71 on enzyme activities of acetylcholinesterase (AChE), lipid peroxidation (LPO), catalase (CAT), glutathione-S-transferase (GST) and protein content on teleostean fishes.	Ecotoxicology and environmental safety (2014), Vol. 107, pp. 120-5	Cellular level endpoints cannot be related to the ecotoxicology Annex I renewal risk assessment.	The RMS agrees with the applicant's reasoning and conclusion given that no link with effect on individual/population is possible based on this paper..
Samanta P. et al.	2014	Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes.	BioMed research international (2014), Vol. 2014, pp. 425159	Cellular level end-points cannot be related to the ecotoxicology Annex I renewal risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Samanta P. et al.	2019	Assessment of adverse impacts of glyphosate-based herbicide, Excel Mera 71 by integrating multi-level biomarker responses in fishes	International Journal of Environmental Science and Technology (2019), Vol. 16, pp. 6291-6300	Molecular level endpoints, associated with biomarker responses are not part of the ecotoxicology risk assessment for fish for an Annex I renewal of a plant protection product in the EU. Observations are not relatable to the EU renewal of glyphosate. Not representative formulation.	The RMS agrees with the applicant's reasoning and conclusion.
Sanchez J. A. A. et al.	2017	Effects of Roundup formulations on biochemical biomarkers and male sperm quality of the livebearing Jenynsia multidentata.	Chemosphere (2017), Vol. 177, pp. 200-210	Formulations contain POEA and therefore not relevant to the EU level ecotoxicological risk assessment for Annex I renewal.	Sanchez et al 2017 states that « It is known that the surfactant MON 0818, containing POEA, integrates the Roundup Original formulation. The MON 0818 (a code of Monsanto for

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					designation for preparation of POEA) is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids and is added to facilitate glyphosate penetration into the plants." This indicates the presence of POEA in the formulation tested. POEA is not authorized in plant protection products containing glyphosate (European Commission, August 2016). Due to presence of POEA, the study is considered not relevant by RMS
Sani A. et al.	2016	Acute toxicity of herbicide (glyphosate) in Clarias gariepinus juveniles.	Toxicology reports (2016), Vol. 3, pp. 513-515	No information on test substance and test design not recognised. Fish too big for use in study.	The RMS agrees with the applicant's reasoning and conclusion.
Santadino M. et al.	2014	Glyphosate Sublethal Effects on the Population Dynamics of the Earthworm Eisenia fetida (Savigny, 1826)	Water, air, and soil pollution (2014), Vol. 225, No. 12, pp. 2207	Classified as relevant but supplementary (EFSA GD Point 5.4.1 - relevance category B)	The objective of this study was to determine the chronic, sublethal toxic effects of glyphosate in its commercial presentation as Roundup® (Monsanto, SL at 48 %) on populations of E. fetida and to evaluate the ecological importance of those effects on earthworms' demographic dynamics. The description of the formulation is not detailed and did not allow to confirm either the presence or absence of POEA no information is provided on the surfactants ofin the formulation used. The study is less relenvant but supplementary. Moreover the experimental design is not sufficiently described to assess its reliability. Endpoints that could be

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					used for risk assessment are not reported. RMS considers this study not reliable and cannot be taken into account for the assessment of the active substance glyphosate itself. The study is considered less relevant but supplementary and not reliable.
Santos S. A. et al.	2019	DIFFERENTIAL TOLERANCE OF CLONES OF Eucalyptus grandis EXPOSED TO DRIFT OF THE HERBICIDES CARFENTRAZONE- ETHYL AND GLYPHOSATE	PLANTA DANINHA (2019), Vol. 37	The study is considered not relevant as it is conducted with Roundup Original. Despite the content being 360 g a.e./L, this product in brazil is based on MON 78087, which contains MON 0818 which is a surfactant system containing POEA. This is not a relevant surfactant for the Annex I submission and therefore data generated using this formulation is not relevant to the EU Annex I renewal process from an ecotoxicology perspective.	The RMS does not agree with the applicant. It is not stated in the report that the formulation contains POEA. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Santos S. A. et al. 2019
Santric L. et al.	2016	Effects of herbicides on growth and number of actinomycetes in soil and in vitro.	Pesticidi i Fitomedicina (2016), Vol. 31, No. 3/4, pp. 121-128	No endpoints presented that can be used in an EU level ecotoxicology risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Santric L. et al. 2016
Santric L. et al.	2018	The effects of nicosulfuron and glyphosate on microbial Activity of different soils	PLANTA DANINHA (2018), Vol. 36	Measured parameters are not used in the EU level ecotoxicology assessment for Annex I renewal. It cannot be confirmed that the product sued was the representative formulation.	The RMS does not agree with the applicant's reasoning and conclusion. This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Santric L. et al. 2018
Saska P. et al.	2016	Treatment by glyphosate-based herbicide alters life history parameters of the rose- grain	Scientific reports (2016), Vol. 6, pp. 27801	5.4.1 case b) Relevant but supplementary information: The paper does not present endpoints that could be used in an EU level ecotoxicological regulatory risk assessment. A formulation was	A test of Roundup Aktiv (Monsanto, Antwerp, Belgium) on rose-grain aphid Metopolophium dirhodum was

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		aphid Metopolophium dirhodum.		tested that is not the representative formulation for the glyphosate EU renewal (the representative formulation is MON 52276).	made to assess lethal and sublethal effects after the herbicide application. The LC50 of the IPA salt of glyphosate on M. dirhodum was equivalent to 174.9 mmol.dm−3 of the active ingredient (CI95: 153.0, 199.0). The population parameters were significantly negatively affected by herbicide application. This LC50 cannot be directly used in the a standard risk assessment. RMS make an attempt to convert this LC50 in LR50 assuming 200 l of water applied per ha. This would result in LR50 of 418 g /ha. However this cannot be used as reliable endpoint. Besides the dosing of the aphids also depends of the volume of water applied on the petri dishes. So the endpoint is uncertain. An other formulation (not the EU representative one) was used. Toxicity of the glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant system in the product. Overall, the study is considered less relevant but supplementary and not reliable.
Saska P. et al.	2017	Treating Prey With Glyphosate Does Not Alter the Demographic Parameters and Predation of the Harmonia axyridis (Coleoptera: Coccinellidae).	Journal of economic entomology (2017), Vol. 110, No. 2, pp. 392	5.4.1 case b) Relevant but supplementary information: Exposure was performed via treated prey, which does not correspond to an adequate route of exposure regarding current test guideline for non- target-arthropods. 2 mL test solution was applied to 50 aphids placed on a filter paper in a petri dish, (dimension unknown). There is no analytical verification, and the study does not conform to guidelines nor GLP. The study is well	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				documented, but no endpoints could be derived which can be applied for the risk assessment. Therefore, the study is considered as supplementary only.	
Saunders L. E. et al.	2013	Root-zone glyphosate exposure adversely affects two ditch species.	Biology (2013), Vol. 2, No. 4, pp. 1488-96	The author describes a formulation that is not the representative formulation for the Annex I.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Saunders L. E. et al. 2013
Schwan-Stoffel A. V. et al.	2012	The effect of herbicides on the germination of urediniospores of Phakopsora Pachyrhizi SYD. & P. SYD. Original Title: Germinacao de Phakopsora Pachyrhizi SID. & P. SID. Sob diferentes herbicidas.	Arquivos do Instituto Biologico Sao Paulo (2012), Vol. 79, No. 3, pp. 381	5.4.1 case b) Relevant but supplementary information: Study describes the impacts of glyphosate on germination of plant pathogen spores.	This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Schwan-Stoffel A. V. et al. 2012
Seguin A. et al.	2017	Sub-lethal effects of a glyphosate- based commercial formulation and adjuvants on juvenile oysters (Crassostrea gigas) exposed for 35days.	Marine pollution bulletin (2017), Vol. 117, No. 1-2, pp. 348-358	Contains POEA, therefore not relevant to EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Shaker B. K. et al.	2018	Effect of exposure to glyphosate pesticide, cadimum and chromium on biomass of algae (*Chlorococcum humicola* and *chlorella vulgaris*) in polluted aqueous culture.	Indian Journal of Public Health Research and Development (2018), Vol. 9, No. 10, pp. 708-713	End-points are based on exposure to both glyphosate and metals and therefore is considered a mixture. Therefore not relevant to EU level risk assessment for glyphosate renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Sharifi Y. et al.	2015	Biodegradation of glyphosate herbicide by Salinicoccus spp isolated from Qom Hoze-soltan lake, Iran	Environmental Health Engineering and Management Journal (2015), Vol. 2, No. 1, pp. 31-36	Paper discusses the potential use of a bacterial strain for biodegrading of glyphosate in a freshwater lake in Iran. Not relevant to the Annex I renewal process in the EU.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Sheehan N. et al.	2018	Glyphosate-containing herbicide impacts physical and behavioral changes during head regeneration in Dugesia (Girardia) tigrina	Bios (2018), Vol. 89, No. 1, pp. 14-22	Formulation used is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Sheehan N. et al. 2018
Shimina V. S. et al.	2010	Effect of Herbicide Excel Mera-71 (Glyphosate) Treatment on Seed Germination and Early Seedling Growth of Black Gram (Vigna mungo, Hepper.) var. T-9.	Advances in Plant Sciences (2010), Vol. 23, No. 2, pp. 515-518	Conducted in India, GLY product used to look at the toxicity to germination, seedling growth in seedlings of black gram. Endpoint not relevant for the regulatory renewal of GLY.	The RMS does not agree with the applicant's reasoning and conclusion. Data gap: Provide the report together with a study summary and a detailed assessment of reliability for the paper of Shimina V. S. et al. 2010
Shiogiri N. S. et al.	2010	Ecotoxicity of glyphosate and aterbane (R) br surfactant on guaru (*Phalloceros caudimaculatus*).	Acta Scientiarum Biological Sciences (2010), Vol. 32, No. 3, pp. 285	5.4.1 case b) Relevant but supplementary information: Conducted in Brazil, looking at comparison of toxicity of glyphosate products with different amounts of surfactant to different fish species and impact on electrical conductivity, dissolved oxygen and pH.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Shiogiri N. S. et al. 2010
Shiogiri N. S. et al.	2012	Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, Piaractus mesopotamicus.	Environmental toxicology and pharmacology (2012), Vol. 34, No. 2, pp. 388-396	Formulations contain POEA and therefore not relevant to the EU level ecotoxicology risk assessment for Annex I renewal.	It is unclear for the RMS why the applicant indicated that the formulation tested contains POEA. Data gap : Provide justification that the formulation used in the paper of Shiogiri N. S. et al. 2012 contains POEA.
Sikorski L. et al.	2019	The effects of glyphosate-based herbicide formulations on Lemna minor, a non-target species	Aquatic Toxicology (2019), Vol. 209, pp.70-80	Difficult to relate the findings to an EU level risk assessment for Annex I renewal as the study was conducted on a non-EU tree species.	The RMS does not agree with the applicant's reasoning and conclusion. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Sikorski L. et al. 2019
Siddhapara M. R. et al.	2012	Toxicity of some commonly used insecticides/herbicides on Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae).	Journal of Biological Control (2012), Vol. 26, No. 3, pp. 251	5.4.1 case b) Relevant but supplementary information: The source of the beetles used was not adequately described. The source and purity of the glyphosate test substance was not described, preventing confirmation ofthe	The RMS does not agree with the applicant's reasoning and conclusion. Data gap: Provide a study summary and a detailed assessment of reliability

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				exposure concentrations used in the test. There was insufficient description of the test system to enable comparison with existing test guidelines to establish acceptability of the approach used. Analytical verification of the exposure concentrations was not performed. No endpoint can be derived from the study. The sudy is considered as supplementary only.	for the paper of Siddhapara M. R. et al. 2012
Sihtmaee M. et al.	2013	Ecotoxicological effects of different glyphosate formulations	Applied soil ecology (2013), Vol. 72, pp. 215	5.4.1 case b) Relevant but supplementary information: The study design and overall conduct were well described. The D. magna toxicity test was performed according to OECD guideline 202 but validity criteria were not mentioned. Analytical verification of the test materials and exposure concentrations within the study was also lacking. Overall, the study is considered to be of limited relevance to the EU annex renewal of glyphosate as the D. magna toxicity test was only a small part of the study, and the soil portion of the study was conducted using exaggerated soil concentrations (up to 1000 times relevant levels). For these reasons, the study is considered supplemental only.	The RMS does not agree with the applicant's reasoning and conclusion. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Sihtmaee M. et al. 2013
Silveira T. et al.	2019	Roundup® Herbicide Decreases Quality Parameters of Spermatozoa of Silversides Odontesthes Humensis	Bulletin of Environmental Contamination and Toxicology (2019), Vol. 102, No. 1	Observations cannot be related to an ecotoxicology risk assessment for EU renewal of glyphosate.	The effect of of Roundup on spermatozoa of the fish silverside (Odontesthes humensis) was investigated after acute exposure at concentrations of 7.8 mg L−1 (a.e.) of glyphosate (in Roundup formulation). Effects were: -a significant decrease in concentration, total and progressive motility, average path distance, straight line distance, path average velocity, curved line velocity, straight line velocity linearity, wobble, amplitude of lateral head

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					displacement, cross beat frequency, and motility period of silverside spermatozoa -increase in membrane fluidity, ROS production and lipid peroxidation -decrease in the mitochondrial functionality This study suggests that Roundup is able to cause losses in several sperm quality parameters, consequently decreasing the fertilization potential of O. humensis spermatozoa. RMS considers these parameters relevant for the risk assessment. However the concentrations tested in laboratory conditions was high (7.8 mg acide equivalent/L) and above those expected in environmentally realistic conditions, so the study is not considered relevant. No intermediate concentration was tested. In view of the formulation description, the use of the results to assess the toxicity of glyphosate as formulated in MON52276 is questionable. In the present study, the authors hypothesized that "the elevated level of polyethoxylated tallow amine (POEA) surfactant in Roundup formulation may act solubilizing the lipids from plasma membrane leading to an increase of membrane fluidity". While presence of POEA in the tested formulation is not clearly stated, RMS assumes its presence very likely as part of the

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					study authors discussion and considers the study not relevant. The study is considered not relevant by RMS.
Simoes T. et al.	2019	Fate and effects of two pesticide formulations in the invertebrate Folsomia candida using a natural agricultural soil.	The Science of the total environment (2019), Vol. 675, pp. 90-97	The formulation used (MONTANA) contains POEA which is not relevant to the EU level ecotoxicology risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Simoes T. et al.	2018	An integrative omics approach to unravel toxicity mechanisms of environmental chemicals: effects of a formulated herbicide	Scientific Reports (2018), Vol. 8, No. 1, pp. 1- 12	Study investigates the impact of a glyphosate formulation that contains POEA on Folsomia. This is not the formulated product for the renewal and POEA is no longer used in the EU.	The RMS agrees with the applicant's reasoning and conclusion.
Siti Hanisah Zahuri et al.	2014	Toxicity testing of three commonly used herbicides on soil-dwelling ant (Family: Formicidae - Odontomachus simillimus).	Borneo Journal of Resource Science and Technology (2014), Vol. 4, No. 1, pp. 28-33	Review, secondary infomation.	While being a review it contains information on toxicity to ant that may be considered for their relevance and reliability by investigating the original report cited in the review. Data gap : Provide full-text articles, summaries and assessment of relevance and reliability of the papers cited in Siti Hanisah Zahuri et al. 2014 that may provide information on effects to ant as non taget species.
Smedbol E. et al.	2018	Effects of low concentrations of glyphosate-based herbicide factor 540A® on an agricultural stream freshwater phytoplankton community	Chemosphere (2018), Vol. 192, pp. 133-141	Test substance not glyphosate or itsmetabolites. Paper presented based on a formulation of glyphosate that is not the representative formulation being considered for the Annex I renewal.	Factor 540® was used. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. The study is considered less relevant but supplementary (formulation issue). Effects of the exposure (96 h) of a phytoplankton community collected in an agricultural stream to various glyphosate concentrations (1, 5, 10,

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					50, 100, 500 and 1000 µg/L*) of Factor 540® GBH were investigated. Typing errors are noted (concentrations are wrongly expressed as g/L instead of µg/L in the text). - The lowest GBH concentration of 1 µg/L reduced chlorophyll a and carotenoid contents. - Low glyphosate concentrations, such as 5 and 10 µg/L, promoted changes in the community's structure and reduced the diversity of the main algal species. - From 50 to 1000 µg/L, the phytoplankton community's composition was modified and new main species appeared. - The highest glyphosate concentrations (500 and 1000 µg/L) affected the shikimate content, the lipid peroxidation and the activity of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase). This study suggests that Factor 540® GBH can modify structural and functional properties of freshwater phytoplankton communities living in streams located in agricultural areas at low glyphosate concentrations. No analytical verification was made. There is no information on environmental conditions (e.g. oxygen, nutrient levels). The variation

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					in the composition in the community between replicates is not shown which makes it difficult to assess the magnitude of the effect. It is not known how the communities look like at the start of the study (sampling at 96h only). There is only a comparison with the 96h-changed control treatment. A sample at t=0 is missing. It is also noted that glyphosate concentration originally present in the stream was evaluated at 1 g/L (±0.02 g/L). While assuming the unit is wrongly expressed in g/L, the presence of glyphosate renders the outcome of the study doubtful. RMS considers that this study is less relevant but supplementary (different formulation tested) and not reliable.
Smedbol E. et al.	2017	Phytoplankton growth and PSII efficiency sensitivity to a glyphosate-based herbicide (Factor 540(®)).	Aquatic toxicology (2017), Vol. 192, pp. 265-273	Achieved end-points are not relatable to an EU level ecotoxicology risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Smedbol E. et al. 2017
Smith C. M. et al.	2019	Developmental and epigenetic effects of Roundup and glyphosate exposure on Japanese medaka (Oryzias latipes).	Aquatic toxicology (2019), Vol. 210, pp. 215-226	Single application rates for glyphosate active substance considered. No specific endpoints presented that could be applied to an EU level ecotoxicology risk assessment for Annex I renewal.	The study examined developmental teratogenic effects and adult-onset reproductive effects of exposure to environmentally relevant concentrations of glyphosate and Roundup in Japanese medaka fish (Oryzias latipes). Hd-rR strain medaka embryos were exposed to 0.5 mg/L glyphosate, 0.5 mg/L and 5 mg/L Roundup (glyphosate acid equivalent) for the first 15 days of their embryonic life and then allowed to sexually mature without further

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					exposure. Whole body tissue samples were collected at 15 days post fertilization (dpf) and brain and gonad samples were collected in mature adults. Hatching success and phenotypic abnormalities were recorded up until 15 dpf. -Roundup (0.5 mg/L) and glyphosate decreased cumulative hatching success, while glyphosate exposure increased developmental abnormalities in medaka fry. -Expression of the maintenance DNA methyltransferase gene Dnmt1 decreased, whereas expression of methylcytosine dioxygenase genes (Tet1, Tet2 and Tet3) increased in fry at 15 dpf suggesting that epigenetic alterations increased global DNA demethylation in the developing fry. -Fecundity and fertilization efficiency were not altered due to exposure. -Among the reproduction-related genes in the brain, kisspeptin receptor (Gpr54-1) expression was significantly reduced in females exposed to 0.5 mg/L and 5 mg/L Roundup, and Gpr54-2 was reduced in the 0.5 mg/L Roundup treatment group. No change in expression of these genes was observed in the male brain. -In the testes, expression of Fshr and Arα was significantly reduced in medaka exposed to 0.5 mg/L Roundup and glyphosate, while the expression of Dmrt1 and Dnmt1 was reduced in

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					medaka exposed to 0.5 mg/L glyphosate. No change in expression of these genes was observed in the ovaries. RMS does not consider the results for Roundup relevant (surfactants may induce effects that cannot be discriminated). Results for glyphosate in isolation are relevant. Overall for glyphosate, while there was no significant difference in reproductive mRNA expression levels in adult medaka ovary samples, a significant decrease in Fshr and Arα mRNA levels was observed in adult testes exposed to glyphosate treatment group, along with a significant decrease in Dmrt1 mRNA levels in the testes of medaka exposed to glyphosate. These results suggest that glyphosate affect the male reproductive system by modulating genes required for spermatogenesis. RMS notes that hatching success was 58.7% in the control. This seems rather low even it has to be acknowledged that no standardized guideline/validity criteria was used to decide whether this rate is acceptable or not. The condition of the fish is questionable. RMS notes that the present study showed an increased incidence of developmental abnormalities in medaka fry chronically exposed to 0.5

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					mg/L glyphosate, suggesting potential teratogenic effects. Systemic toxicity cannot be discarded. Analytical verification was not made. The decrease in Fshr and Arα mRNA levels observed in adult testes for glyphosate treatment group at 0.5 mg/L was not observed for Roundup at 5 mg/L. This is also the case for the decrease in Dmrt1 mRNA levels in the testes of medaka exposed to glyphosate (no decreased for Roundup at 5 mg/L). The study authors hypothesized that glyphosate has the ability to display non-monotonic dose-responses taking the shape of a U-shaped curve (and that in the present study, the lack of response at 5 mg/L Roundup would actually correspond to an intermediate dose levels). The number of tested concentrations are not sufficient to support this. RMS doubts the reliability of the conclusions of this study. The results from this study (for glyphosate) are relevant for the assessment of potential for endocrine disruption. The study results are considered not reliable..
Song H.	2010	Toxic action of acetamiprid, glyphosate and their combined pollution on Hydra magnipapillata	Anhui Nongye Kexue (2010), Vol. 38, No. 20, pp. 10811	5.4.1 case b) Relevant but supplementary information: Test species (freshwater polyp) collected from a rural pond in China. It is not clear what previous exposure the test species may have had to pesticides. It is not clear if the glyphosate is technical grade or product; the concentrations are from 0.14 to 36 mg/L.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Song H. 2010 (Toxic action of acetamiprid, glyphosate and their combined pollution on *Hydra magnipapillata*)

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Song H. et al.	2010	The Single and Binary-Combined Acute Toxicities of Five Common Pesticides on Hydra Magnipapillata	Journal of Anhui Normal University (Natural Science) (2010), Vol. 33, no. 2, pp. 159	5.4.1 case b) Relevant but supplementary information: Test species (freshwater polyp) collected from rural pond in China, it is not clear what exposure the test species may have had to pesticides or other chemicals previously. It is not clear if the glyphosate is technical material or product; the concentrations are from 40 to 227 mg/L.	Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Song H. et al. 2010 (The Single and Binary-Combined Acute Toxicities of Five Common Pesticides on *Hydra Magnipapillata*)
Sribanjam S. et al.	2018	Toxic effects of the herbicide glyphosate on enzymes activities and histopathological changes in gill and liver tissue of freshwater fish, Silver barb (Barbonymus gonionotus)	Bioscience Research (2018), Vol. 15, No. 2, pp. 1251-1260	Data presented cannot be related to an EU level ecotoxicology risk assessment for Annex I renewal purposes. Roundup contains POEA which is not present in the representative formulation for the renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The presence of POEA is not stated in the report. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Sribanjam S. et al. 2018
Stecca C. S. et al.	2016	Side-Effects of Glyphosate to the Parasitoid Telenomus remus Nixon (Hymenoptera: Platygastridae).	Neotropical entomology (2016), Vol. 45, No. 2, pp. 192	5.4.1 case b) Relevant but supplementary information: The study was conducted in accordance with the protocols proposed by IOBC. Exposure via overspray on egg-cards and parasitoid pupae does not correspond to an adequate route of exposure according to current guidelines for testing non-target arthopods. The test design for the bioassay where adults are exposed to dry residues moderately described. The mortality of parasitoids during exposure is unclear, however, the spray deposit is given. The assessment of the biological endpoints in not precisely reported; day of emergence of parasitoids is not given. As the biological data do not report results in values useful for the risk assessment, there is no analytical verification, and the study is non GLP, the study can be considered as supplementary only.	None of the formulations was classified as harmful or moderately harmful to this parasitoid when exposure occurred at the pupal or adult stages. However application rates were not sufficient to cover all uses intended. The study does not provide relevant data to be used in the risk assessment.
Stefanello Junior G. J. et al.	2011	Selectivity of herbicides registered for corn at the immature stages of	Planta Daninha (2011), Vol. 29, pp. 1069-1077	Test species was parasitoid T. pretiosium in different immature stages (egg-larva, pre-pupal and pupal stages). However, the test solutions	The RMS agrees with the applicant’s reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		Trichogramma pretio sum (Hymenoptera: Trichogrammatidae). Seletividade de herbicidas registrados para a cultura do milho aos estadios imaturos de Trichogramma pretiosum (Hymenoptera:		were sprayed onto egg-cards containing the parasitised eggs. Therefore this is not an adequate route of exposure and thus not relevant for the risk assessment.	
Stellin F. et al.	2017	Effects of different concentrations of glyphosate (Roundup 360A®) on earthworms (Octodrilus complanatus, Lumbricus terrestris and Aporrectodea caliginosa) in vineyards in the North-East of Italy	Applied soil ecology (2018), Vol. 123, pp 802	5.4.1 case b) Relevant but supplementary information: The study has not been conducted according to a recognized test guideline and there are no validity criteria presented.	The co-formulants are not stated but the study author noted that Roundup360® was made with a polyethoxylated tallow amine surfactant that is more toxic than glyphosate itself. POEA is not authorized in plant protection products containing glyphosate (European Commission, August 2016). Some effects were observed but it is not possible to discriminate between glyphosate and POEA. Due to presence of POEA, the study is considered not relevant by RMS. Reliability was not assessed.
Stenoien C. et al.	2018	Monarchs in decline: a collateral landscape-level effect of modern agriculture. Special Section: The impact of transgenic crops on protected arthropods.	Insect Science (2018), Vol. 25, No. 4, pp. 528-541	Concerns a review of the decline of monarch butterflies in the US. Not relatable to an EU level assessment for Annex I renewal.	RMS consider that the study may provide information useful for assessment of biodiversity. Data gap : Provide a summary and assessment of relevance and reliability of the paper of Stenoien C. et al. 2018, particularly regarding the information that could be used for assessment of impact on biodiversity.
Sun K.-F. et al.	2013	Ecological risks assessment of organophosphorus pesticides based on response of Scenedesmus quadricanda.	China Environmental Science (2013), Vol. 33, No. 5, pp. 868-873	Endpoints not relevant to an EU level ecotoxicology risk assessment as they are not relatable.	This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability

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					for the paper of Sun K.-F. et al. 2013 (Ecological risks assessment of organophosphorus pesticides based on response of *Scenedesmus quadricanda*)
Sun K.-F. et al.	2013	Ecological risks assessment of organophosphorus pesticides on bloom of Microcystis wesenbergii	International biodeterioration & biodegradation (2013), Vol. 77, pp. 98-105	Endpoints measured are not relevant or relatable to an EU level Annex I ecotoxicological risk assessment.	Hormetic response may increase competitive advantage to overcome other species. Therefore this paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Sun K.-F. et al. 2013 (Ecological risks assessment of organophosphorus pesticides on bloom of *Microcystis wesenbergii*)
Sun Q. et al.	2012	Effects of typical herbicides on soil respiration and N2O emissions from soil added with different nitrogen fertilizers.	Huan jing ke xue= Huanjing kexue (2012), Vol. 33, No. 6, pp. 1994	5.4.1 case b) Relevant but supplementary information: The study uses soil from fields in China, without describing the history of the fields (e.g. prior pesticide and fertilizer use), soil sampling, and soil storage conditions prior to the start of the experiment. Soil characteristics are unclear as no information on e.g. CEC and water holding capacity is available. The study was not conducted to a relevant guideline and thus no validity criteria are available. A negative control was included, but no information on replicates is available and only one test item concentration was tested. No positive control was tested. Application of the test item is not described well, the active substance content of the test item is not given and no verification of applied test amount was performed. Finally, there is no quantifiable endpoint presented.	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Sushilkumar et al.	2017	Herbicides effect on fish mortality and water quality in relation to chemical control of alligator weed.	Indian Journal of Weed Science (2017), Vol. 49, No. 4, pp. 396-400	Methods and end-points not relatable to EU level ecotoxicology assessment for renewal purposes.	The RMS agrees with the applicant's reasoning and conclusion.
Sushilkumar et al.	2017	Chemical control of duck weed and its effect on water quality and residue.	Indian Journal of Weed Science (2017), Vol. 49, No. 1, pp. 105-107	Difficult to relate observed findings to an EU level risk assessment for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Szemeredy G. *et al.*	2016	Toxicity test of individual and combined toxic effects of herbicide GLIALKA STAR and lead acetate on chicken embryos. Original Title: GLIALKA STAR gyomirto szer es az olom acetat egyedi es interakcios toxicitasanak vizsgalata madarembriokban.	Novenyvdelem (2016), Vol. 52, No. 10, pp. 483 487	Formulation tested via injection to chicken embryos. This dosing approach does not represent a typical route of exposure. The tested formulation GLIALKA STAR is not the EU representative formulation for the glyphosate EU renewal. Furthermore, in the absence of a concurrent control for each of the component of the formulation, it is not possible to conclude whether the observed effects claimed to be secondary to exposure to glyphosate are due to glyphosate exposure or to one of the other components.	We agree that the exposure pathway (injection into embryos) is not representative of field conditions and hence this study is not relevant for the risk assessment. Further, due to the significant embryo mortality it seems that the treatment levels were above the MTC (maximum tolerable concentration), and therefore the results are also less useful for the ED assessment.
Tahir H. M. et al.	2019	Effect of Pesticides on Biological Control Potential of Neoscona theisi (Araneae: Araneidae)	Journal of insect science (2019), vol. 19, no. 2, pp. 1	5.4.1 case b) Relevant but supplementary information: Considered supplemental as the approach used does not follow an approach recognised at EU level for use in ecotoxicological regulatory risk assessment.	Commercial product was used but the specifications of the pesticide products used in the test were not stated beyond the active substance name. Glyphosate was used at the rate of 100 ml/100 liters per acre however density of the test material is unknown and no conversion in adequate dose rate is feasible. Moreover, no biological data is available. This study does not follow standardized guideline. The parameters investigated are relevant but the results are considered not reliable for regulatory risk assessment purpose.
Tang Y. et al.	2014	The influence of three different types of herbicides on biodiversity	Advanced Materials Research (2014), Vol. 838-	Information presented is not directly relevant to the ecotoxicology risk assessment for Annex I renewal	This paper may provide relevant information for the indirect effects/biodiversity assessment.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
			841, pp. 2417-2426		Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Tang Y. et al. 2014
Tapkir S. D. et al.	2019	Impact, recovery and carryover effect of Roundup® on predator recognition in common spiny loach, Lepidocephalichthys thermalis.	Ecotoxicology (2019), Vol. 28, No. 2, pp. 189-200	The formulation is not the representative formulation being used for the Annex 1. Its identity cannot be confirmed. The observed effects based on reactions to non-specific alarms / cues cannot be related to an EU level ecotoxicology risk assessment for Annex I renewal.	This study is considered not directly relevant for the risk assessment but still relevant for the investigation of "other types of effects" not currently covered by the risk assessment scheme. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Tapkir S. D. et al. 2019
Tkaczuk C. et al.	2016	The influence of selected pesticides on the growth of entomopathogenic fungi from the entomophthoralean order (Entomophthorales). Wpyw wybranych srodkow ochrony roslin na wzrost grzybow owadobojczych z rzedu owadomorkowcow (Entomophthorales).	Annales Universitatis Mariae Curie-Skodowska. Sectio E, Agricultura (2016), Vol. 71, No. 1, pp. 65-75	Test design not relevant to an EU level ecotoxicology risk assessment to support Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Tome H. V. V. et al.	2020	Frequently encountered pesticides can cause multiple disorders in developing worker honey bees.	Environmental pollution (2020), Vol. 256, pp. 113420	5.4.1 case b) Relevant but supplementary information: The data presented are relevant to the wider discussion of the effects of glyphosate on pollinators, but as the rates established for glyphosate used in the study were based on reported levels found in pollen and wax from another active substance, from an exposure perspective, they cannot be related to glyphosate.	The RMS does not agree with the applicant's reasoning and conclusion. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Tome H. V. V. et al. 2020
Topal A. et al.	2015	Effects of glyphosate on juvenile rainbow trout (Oncorhynchus mykiss): transcriptional and enzymatic analyses of antioxidant defence	Ecotoxicology and environmental safety (2015), Vol. 111, pp. 206-14	Cellular and molecular level results, not considered relevant to EU level ecotoxicology risk assessment.	Histopathological liver damage and swimming performance are considered relevant parameters. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Topal A. et al. 2015

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		system, histopathological liver damage and swimming performance.			
Triana Velasquez T. M. *et al.*	2013	Lethal and Sublethal Effects of Glyphosate (Roundup® Active) to Embryos of Colombian Anurans. Original Title: Efectos letales y subletales del glifosato (Roundup® Activo) en embriones de anuros colombianos.	Acta Biologica Colombiana (2013), Vol. 18, No. 2, pp. 271-278	The formulation data presented in the paper are for a formulation that is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered less relevant but supplementary and reliable with restrictions. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Truta E. et al.	2011	Evaluation of Roundup-induced toxicity on genetic material and on length growth of barley seedlings.	Acta biologica Hungarica (2011), Vol. 62, No. 3, pp. 290	5.4.1 case b) Relevant but supplementary information: Impact of glyphosate product on barley seedling development. Unclear how endpoint could be used in risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Uchida M. et al.	2012	Toxicity evaluation of glyphosate agrochemical components using Japanese medaka (Oryzias latipes) and DNA microarray gene expression analysis	The Journal of toxicological sciences (2012), Vol. 37, No. 2, pp. 245	5.4.1 case b) Relevant but supplementary information: The material and methods part lack some important information. Only glyphosate was sufficiently documented, but the formulation Roundup is not specified. In addition, it is unclear whether the test concentrations for the formulation refer to the active ingredient or to the product. The test design is not adequately described. Only a concentration range was given and tested dose rates remain unclear. The performance of a control group as well as the description of observations is not reported. No mortality data neither for the test concentrations nor for the controls was given to evaluate the results. Furthermore, there was no analytical verification of test concentrations reported. No suitable exposure throughout the test was demonstrated and thus the reliability of the study is questionable. The test guideline followed was not stated nor was the study conducted to GLP.	The RMS agrees with the applicant's reasoning and conclusion.
Udeh G. N. et al.	2014	Acute toxicity of Delsate® herbicide (glyphosate) on albumin and blood	Journal of Aquatic Sciences (2014), Vol. 29, No. 2, pp. 309-315	Test item is a formulation other than the representative formulation.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		urea nitrogen of African catfish, Clarias gariepinus (Burchell, 1822).			itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Udeh G. N. et al. 2014
Udeh G. N. et al.	2014	Behavioural and some physico-chemical assessment of fresh water catfish Clarias gariepinus (Burchell, 1822) exposed to acute concentrations of Delsate® herbicide (glyphosate).	Journal of Aquatic Sciences (2014), Vol. 29, No. 2, pp. 275-283	Contains a surfactant system that is not relevant to the EU level ecotoxicology risk assessment for renewal of MON 52276 onto Annex I.	Delsate contains 18% tallow amine. It is not clearly stated whether it is POEA or not. Therefore the study is considered "less relevant but supplementary". Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Udeh G. N. et al. 2014
Ujszegi J. et al.	2015	No observable effect of a glyphosate- based herbicide on two top predators of temporal water bodies.	Environmental toxicology and chemistry (2015), Vol. 34, No. 2, pp. 307-13	The test substance contains polyethoxylated tallowamine surfactant, which is not allowed in herbicidal formulations in the EU. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Ujszegi J. et al.	2016	No effect of a glyphosate-based herbicide on larval dragonflies (aeshna cyanea) and adult newts (lissotriton vulgaris) in a laboratory-based Experiment.	Acta Zoologica Academiae Scientiarum Hungaricae (2016), Vol. 62, No. 4, pp. 355-367	The test substance contains polyethoxylated tallowamine surfactant, which is not allowed in herbicidal formulations in the EU. Due to the test materials not being the representative formulation for the EU renewal, the study is not relevant to the EU level Annex I ecotoxicology risk assessment.	The RMS does not agree with the applicant's reasoning and conclusion. The presence of POEA is not stated in the report. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Ujszegi J. et al. 2016
Usenko O. M. et al.	2016	Effects of different pesticides on virulence and mortality of some entomopathogenic nematodes.	ISJ-Invertebrate Survival Journal (2016), Vol. 13, pp. 111	5.4.1 case b) Relevant but supplementary information: Nematode mortality and effects on virulence are not endpoints used in EU level ecotox risk assessment for the renewal.	Effects on entomopathogenic nematodes are not in the scope of the ecotoxicological risk assessment.
Vajargah M. F. et al.	2018	Acute toxicity effect of glyphosate on survival rate of common carp, Cyprinus carpio	Environmental Health Engineering and Management Journal (2018), Vol. 5, No. 2, pp. 61-66	Non-representative formulation (Glyphosate Aria 41% SL) was tested. The test conditions throughout the whole exposure period were not documented. No information on source and composition of test media reported. There was no analytical verification of test concentrations reported and the study is not conducted according to a recognised test guideline. No validity criteria	The study is less relevant but supplementary due to formulation issue. The study may provide relevant data for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Vajargah M. F. et al. 2018

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				are stated so the validity of the study cannot be confirmed The size of the fish used in the test results in a fish loading rate of 1.23 g fish/Litre, which exceeds the loading rate required by internationally recognised fish testing guidelines. The testing design is not properly described i.e. whether a static, static renewal or flow through test design was used. The water quality parameters measured would suggest that the environmental conditions were maintained for the exposure duration, although this can also not be confirmed from the information presented. The water temperature being maintained at 26±1 ºC, exceeds the upper temperature limit for testing with Cyprinus carpio (24 ºC).	
Vannini A. et al.	2016	Bioaccumulation, physiological and ultrastructural effects of glyphosate in the lichen Xanthoria parietina (L.) Th. Fr.	Chemosphere (2016), Vol. 164, pp. 233-240	Article discusses the use of lichens as a bioindicator model of glyphosate exposure. Not relatable to an EU level ecotoxicology risk assessment for the renewal of Glyphosate onto Annex I in the EU.	Toxic effects resulting from glyphosate uptake by both soaking and spraying were found at physiological and ultrastructural level, both in the algal and fungal partner, with negative effects being generally both dose- and time-dependent. This paper may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Vannini A. et al. 2016
Vazquez D. E. et al.	2018	Glyphosate affects the larval development of honey bees depending on the susceptibility of colonies	PLoS One (2018), Vol. 13, No. 10, pp. E0205074	5.4.1 case b) Relevant but supplementary information: The method of exposure used for the bees were not described. Endpoints presented are not relatable to an EU level ecotoxicological regulatory risk assessment for the glyphosate EU renewal.	The study aimed to assess the effects of glyphosate on larval development under chronic exposure during in vitro rearing. The study exposed brood with food containing glyphosate (1.25±5.0 mg per litre of food) and investigated larval survival among other paramaeters such as delayed moulting, reduced weight, their variability among colonies. The

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					transcription of immune/detoxifying genes in the guts of larvae exposed to GLY was studied. The brood received a total dose of glyphosate of 137.5, 275 and 550 ng a.e. per treatment. RMS considers the parameters investigated of low relevance except for brood survival. However RMS does not agree with the conclusion of the study as the performance of the control was very variable among colonies. No dose effect relationship was observed. Then it appears likely that reduction of brood survival was not necessarily caused by the treatment. No reliable endpoint can be derived from this study. The study is considered relevant but not reliable for regulatory risk assessment purpose.
Veeraiah K. et al.	2015	Impact of glyphosate on biochemical constituents of the freshwater fish, catla catla	International Journal of Bioassays (2015), Vol. 4, No. 7, pp. 4139-4144	Formulation is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Veeraiah K. et al. 2015
Velasques R. R. et al.	2016	Roundup(®) in Zebrafish: Effects on Oxidative Status and Gene Expression.	Zebrafish (2016), Vol. 13, No. 5, pp. 432	5.4.1 case b) Relevant but supplementary information: The data presented demonstrates that in the presence of a toxicant, there are changes in the oxidative status of zebrafish gills and liver tissue. However, these data cannot be related to an Annex I risk assessment for renewal.	The objective of this study was to determine the effects of Roundup on oxidative status in adult Danio rerio liver and gills. Reactive oxygen species and antioxidant capacity were measured in fish after exposure to Roundup. Furthermore, gene expression related to antioxidant

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					response was evaluated. These parameters are not relatable to the risk assessment and the study is considered not relevant. Not relevant for regulatory risk assessment purpose.
Vera M. S. et al.	2012	Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities.	Ecotoxicology (2012), Vol. 21, No. 7, pp. 1805-16	On review of the paper, the findings of the study conducted in Argentina were difficult to relate to the EU level ecotoxicology risk assessment. The formulation used differs to the representative formulation for the Annex I in the EU.	The study may be considered less relevant but supplementary in view of RMS criteria. Moreover the study investigate the indirect effects on freshwater microbial communities which could be of interest for assessment of biodiversity through indirect effects assessment. Data gap : provide a summary and assessment of relevance and reliability of the paper of Vera M. S. et al. 2012 (Direct and indirect effects of the glyphosate formulation Glifosato Atanor® on freshwater microbial communities) that may be useful for biodiversity assessment.
Vera-Candioti J. et al.	2013	Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live- bearer fish Cnesterodon decemmaculatus (Jenyns, 1842).	Ecotoxicology and environmental safety (2013), Vol. 89, pp. 166-73	Formulations used are not relevant to the Annex I renewal of glyphosate in the EU.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Vera-Candioti J. et al. 2013
Viti M. L. et al.	2019	Translocation and Root Exudation of Glyphosate by Urochloa brizantha and its Transport on Sugarcane and Citrus Seedlings	Planta Daninha (2019), Vol. 37	Paper discusses the translocation potential of glyphosate via the root zone, after application to palisade grass planted in association with sugar-cane. No endpoints relevant for an EU level ecotoxicology assessment.	The RMS agrees with the applicant’s reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Vllasaku I. et al.	2018	Investigation of genotoxic effect of herbicid Randap 480 ec at goldfish (Carassius auratus)	International Journal of Pharmaceutical Sciences Review and Research (2018), Vol. 48, No. 1, pp. 7/1-7/3	Identity of the formulated product used is not the same as the representative formulation being considered for the Annex I.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Vllasaku I. et al. 2018
Voeroes M. et al.	2019	Influence of agro-environmental pollutants on a biocontrol strain of Bacillus velezensis	MicrobiologyOpen (2019), Vol. 8, No. 3, pp. e660	This paper discusses the impact of glyphosate on pesticide resistance strains of biocontrol agents. This is not considered relevant to an EU level ecotoxicology risk assessment for EU Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Vrisman C. M. et al.	2014	Influence of herbicides and fungicides in the carpogenic germination of sclerotia of Sclerotinia sclerotiorum (Lib.) de Bary. Influencia de herbicidas e fungicidas na germinacao carpogenica de esclerodios de Sclerotinia sclerotiorum (Lib.) de Bary.	Bioscience Journal (2014), Vol. 30, No. 2, pp. 477-483	Study describes the impact of multiple pesticides on the germination of Sclerotina scleroteroium - a soil fungus. The end- points are not useable in an EU level Anneix ecotox risk assessment. 400 L/ha (if this were the representative formulation) would be equivalent to 1440 kg/ha significantly higher than any application rate proposed. Therefore this is not relatable to EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Wagner N. *et al.*	2017	Effects of a commonly used glyphosate-based herbicide formulation on early developmental stages of two anuran species.	Environmental science and pollution research international (2017), Vol. 24, No. 2, pp. 1495-1508	Not the representative formulation for the Annex I renewal. Therefore not relevant to EU renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The study is considered relevant and reliable. The study summary and RMS assessment are presented in the Appendix to Vol 3CA B9.
Wang F. et al.	2014	Acute Toxicity and Oxidative Stress of Two Herbicides on Earthworm Eisenia fetida.	Asian Journal of Ecotoxicology (2014), Vol. 9, No. 6, pp. 1210-1218	The achieved acute end-points are not considered relevant to an EU level ecotox risk assessment for renewal purposes.	Agree
Wang Y. et al.	2013	Joint Toxicity of Arsenic, Glyphosate and Dichlorvos to C. elegans.	Asian Journal of Ecotoxicology (2013), Vol. 8, No. 2, pp. 262-267	Mixture study with aresenic.	Agree.

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Wang Y. et al.	2014	Toxicological effects of glyphosate to Pyramidomonas delicatula and Alexandrium tamarense in water environment	Nongyao (2014), Vol. 53, No. 1, pp. 45-48	End-points are not relatable to an EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Wang Y. et al.	2012	Acute Toxicity of Twenty-Two Commonly Used Herbicides to Earthworm (Eisenia fetida).	Asian Journal of Ecotoxicology (2012), Vol. 7, No. 3, pp. 317-325	The achieved acute endpoints are not considered relevant to an EU level ecotoxicology risk assessment for renewal purposes.	Agree
Watts C. et al.	2016	Responses of invertebrates to herbicide in Salix cinerea invaded wetlands: Restoration implications	Ecological management & restoration (2016), Vol. 17, No. 3, pp. 243-249	Non-EU monitoring study. Extrapolation to EU is difficult.	Not specific to glyphosate. Agree
Webster T. M. U. et al.	2015	Global transcriptomic profiling demonstrates induction of oxidative stress and of compensatory cellular stress responses in brown trout exposed to glyphosate and Roundup.	BMC Genomics (2015), Vol. 16, No. 32	No data relevant to the EU data requirements is presented and therefore does not support the EU level renewal of glyphosate.	The RMS agrees with the applicant's reasoning and conclusion.
Wech J. et al.	2018	The effect of willow control using a glyphosate formulation on aquatic invertebrates within a New Zealand wetland	New Zealand journal of marine and freshwater research (2018), Vol. 52, No. 1, pp. 16-41	Multi-year environmental monitoring project conducted in New Zealand. Not relatable to EU level risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Weeks Santos S. et al.	2019	A glyphosate-based herbicide induces sub-lethal effects in early life stages and liver cell line of rainbow trout, Oncorhynchus mykiss.	Aquatic toxicology (2019), Vol. 216, pp. 105291	Non-standard test design and results that cannot be related to an EU level risk assessment for EU renewal purposes.	Parameters directly relevant for the risk assessment were investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Weeks Santos S. et al. 2019
Winnick B. and Dzialowski E. M.	2013	The effects of glyphosate based herbicides on chick embryo morphology during development	FASEB Journal, (2013) Vol. 27, Supp. 1. Meeting Abstracts. Abstract Number: 874.12. Meeting Info: Experimental Biology	This is a conference abstract only. Direct injection of glyphosate based herbicide into fertilized chicken eggs is not a relevant route of exposure, in this invalidated test system.	The RMS agrees with the applicant's reasoning and conclusion. The exposure regimen is not considered comparable to realistic exposure in the field.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
			2013, Boston, United States, April 20 24, 2013.		
Wrinn K. M. et al.	2012	Predator cues and an herbicide affect activity and emigration in an agrobiont wolf spider.	Chemosphere (2012), Vol. 87, No. 4, pp. 390-6	Discusses results of experiments conducted using a formulation of glyphosate that contains POEA. Not relevant to EU risk assessment.	Due to the presence of POEA in the formulation tested, the study is not relevant.
Wu L. et al.	2016	Physiological effects of the herbicide glyphosate on the cyanobacterium Microcystis aeruginosa.	Aquatic toxicology (2016), Vol. 178, pp. 72-9	End-points presented cannot be used in the EU level renewal risk assessment for glyphosate from an ecotoxicology perspective.	Parameters directly relevant for the risk assessment (cell density) were investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Wu L.et al. 2016
Xia S. et al.	2013	Induction of vitellogenin gene expression in medaka exposed to glyphosate and potential molecular mechanism	Zhongguo Huanjing Kexue (2013), Vol. 33, No. 9, pp.1656	5.4.1 case b) Relevant but supplementary information: The study was not conducted according to GLP and a relevant guideline was not followed. The current EU stepwise endocrine approach is detailed, and the approach conducted within this study does conform to the suggested guidance. Significant limitations in the study include a lack of a standard testing approach or specific validation criteria. The test concentrations were not analytically verified and the critical dose regime provided to the Medaka is lacking. Similarly the source of the fish tested is unknown. No clear dose response relationship or derived endpoint from the study could be determined.	Original report in Chinese, results are based on a document translated in english. To demonstrate the estrogenic activities of glyphosate and clarify the underlying molecular mechanism, 1-3 days old Japanese medaka were exposed to 0.2, 2, 20, 200, 2000 μg/L of glyphosate for 5 weeks. Transcription levels of vitellogenin (VTG I) and enzyme genes involved in 17β-E2 biosynthesis and metabolism were measured by Q-RT-PCR. This study states that glyphosate (in formulation) can induce VTG expression in Medaka in a certain concentration range, showing estrogen effect. This was however inhibited at the high glyphosate concentrations (at 200 μg/L and 2000 μg/L for female, at 2000 μg/L for male). Also, there are certain differences in the induction

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					mechanism of estrogen effect between male and female fish. In female fish, glyphosate can up-regulate the expression of FSH to induce the expression of CYP19A (aromatase), thus increasing the ability of estrogen synthesis. However, in male fish, the change of VTG expression was induced by inhibiting estrogen-metabolizing enzymes (CYP1A and CYP3A) in the liver resulting in the increase of the 17 β-E2 concentration in vivo. A formulation was used and co-formulants are not stated. Toxicity of glyphosate-based herbicides to non-target organisms vary within a wide range, depending on the surfactant in the product. The study does not follow a standardized approach. No analytical verification available. No information is provided about the toxicity of the tested doses (no data was available on the mortality, bodyweight, growth, etc…). Then it is not possible to determine whether the effects on protein expression are a result of general systemic toxicity. No biological data presented, only graphs. The study is relevant but of low reliability. It is proposed by RMS to not include it in the assessment of potential for endocrine disruption.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Xie RuiTao et al.	2010	The acute toxicity of five pesticides to yellow catfish Pelteobagrus vachelli.	Fisheries Science (2010), Vol. 29, No. 5, pp. 274	5.4.1 case b) Relevant but supplementary information: No information on the test item whether it was product or active ingredient was provided. Therefore, the biological results can not be used for the ecotoxicological regulatory risk assessment and are seen as unreliable.	Assessment and conclusion by RMS: Original report in Chinese, results are based on a document translated in english. The acute toxicity of glyphosate to yellow catfish Pelteobagrus vachelli juveniles was determined in a static test. Glyphosate had the LC50 of 15.38 mg/L for 24 h, 13.43 mg/L for 48 h, and "safe concentration" of 3.072 mg/L. "Safe concentration" was not defined but it is certainly not a NOEC as its value is lower than the lowest tested dose. The application method (preparation of test solution etc.) is not specified. The concentrations used are unclear (appear to be tested in a range between 7 to 20 mg/L). It is not known if they correspond to nominal or measured concentration. Clear dose-effect relationship was observed, that may be indicate a greater senvity of the catfish species. However no information on the test item whether it was product or active ingredient was provided. The study is relevant but results are seen as unreliable for regulatory risk assessment purpose.
Xu Y. et al.	2010	Acute Toxicity of Ten Pesticides to Larval Red Swamp Crayfish Procambarus Clarkii.	Asian Journal of Ecotoxicology (2010), Vol. 5, No. 1, pp. 50.	5.4.1 case b) Relevant but supplementary information: Effects on red swamp crayfish. Test species raised in and collected from a rice field in Shanghai. It is not clear what exposure the test species may have had to pesticides or other chemicals previously. It is not clear if the	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				glyphopsate is technical or product. No biological results (e.g. mortalities) for the control or any test concentration reported. The study is considered unreliable.	
Xu Y-g. et al.	2015	Joint Toxicity of Glyphosate and As(III)to Daphnia magna in Aquatic Environment	Journal of Agro-Environment Science (2015), Vol. 34, No. 11, pp. 2076	5.4.1 case b) Relevant but supplementary information: This study concentrates on models used to estimate the individual and mixture toxicity of glyphosate and As (III) to Daphnia magna. LC50 values were compared with measured data. The study was not conducted according to GLP, however the acute toxicity studies were conducted to a relevant ISO guideline. Preparation and dose verification were not performed therefore the endpoint is questionable. The study is considered unreliable.	Parameters directly relevant for the risk assessment were investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Xu Y-g. et al. 2015
Yang X. et al.	2019	Effects of the glyphosate-based herbicide roundup on the survival, immune response, digestive activities and gut microbiota of the Chinese mitten crab, Eriocheir sinensis	Aquatic toxicology (2019), Vol. 214, pp. 105243	5.4.1 case c) Relevance cannot be determined: Potential effects to gut microbes are not part of the EU risk assessments. Suitable scientific approaches to assess effects are not specified, thus relevance of the effects remained unclear. This study uses a high dose of roundup formulation. The surfactants in Roundup are known to be toxic to aquatic animals. This publication indicates a potentially significant decline in survivial due to Roundup. Therefore, results obtained for other endpoints beyond survival may be secondary to known toxicity of the surfactants.	In this study, the survival rate, intestinal and hepatopancreatic immune and digestive functions, and the intestinal microbial diversity of Chinese mitten crab (Eriocheir sinensis) were evaluated after 7 days of exposure to a Roundup formulation. One tested dose: 48.945 mg/L. The results showed that roundup significantly affected all the parameters investigated. RMS notes the absence of clear conceptual link between effects on the digestive enzyme activity, the diversity of intestinal microorganisms and the specific protection goals for aquatic invertebrates (SPG). It is agreed that it may play a role in the population health, but such link is not

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					immediate in conceptual terms and not quantifiable. Data on survival were reported (for the only concentration of 48.945 mg/L) but only graphically. This parameter is relevant. From the graph it can be inferred (by RMS) a survival rate of approximately 75-80% for the treated group and above 90% in the control group. The reliability of this result cannot be verified by RMS (no detailed results). RMS highlights that the other parameters investigated in this study do not seem affected to a large extent particularly considering the high concentration that was tested. Then, the study do not provide evidence that such effects would occur under realistic conditions of use (tested concentration was far higher the RAC value). No endpoint can be derived from this study. No specific guideline was followed, no analytical verification was available. Besides, the test substance was identified only as 'Roundup'. The surfactants were not stated. Effects (lethal and sublethal) were observed but it is not possible to discriminate between glyphosate and surfactants. Overall, the study is considered not relevant for regulatory risk assessment and not reliable by RMS.
Yang Z. et al.	2018	Toxic effects of four commonly-used agrochemicals on Arma chinensis and Picromerus lewisi.	Agricultural Biotechnology (2018), Vol. 7, No. 5, pp. 153-155, 158	Test item is a glyphosate formulation that is not the representative fornmulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Yang Z. et al. 2018
Ye J. et al.	2019	The Growth, Apoptosis and Oxidative Stress in Microcystis viridis Exposed to Glyphosate.	Bulletin of environmental contamination and toxicology (2019), Vol. 103, No. 4, pp. 585-589	Achieved end-points are not releatable to an EU level ecotoxicology assessment for Annex I renewal.	This study report was provided in the top-up literature (KCA 9-002). The study investigated the growth, apoptosis and oxidative stress of the cyanobacterium Microcystis viridis exposed to glyphosate. Despite lack of critical data, this study may be informative for this algae group (cyanobacteria) and provide further evidence on its (lesser?) sensitivity. Data gap : Provide a study summary including detailed assessment of relevance and reliability.
You W-y. et al.	2010	Toxicity Evaluation of Sixteen Herbicides to Bombyx mori.	Asian Journal of Ecotoxicology (2010), Vol. 5, No. 1, pp. 91	5.4.1 case b) Relevant but supplementary information: Effects on silkworm via exposure of treated leaves. However, the application method is not specified. The amount of test solution per leaf, the consumed diet per silkworm and the number of organisms per replicate is unclear. Also no control results are available. Therefore the biological results can not be used for risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Yousaf S. et al.	2013	Effect of Pesticides on the Soil Microbial Activity.	Pakistan Journal of Zoology 2013), Vol. 45, No. 4, pp. 1063-1067	Achieved end-points are not relevant to an EU level risk assessment. Novel test design with no positive control and cannot confirm dose.	RMS considers this study may provide relevant data to be used in a Weight of evidence assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Yousaf S. et al. 2013
Yusof S. et al.	2014	Effect of glyphosate-based herbicide on early life stages of Java medaka (Oryzias javanicus): a potential tropical test fish.	Marine pollution bulletin (2014), Vol. 85, No. 2, pp. 49	5.4.1 case b) Relevant but supplementary information: There is insufficient explanation provided on the analytical verification of the test concentrations. It is not clear which Roundup formulation was tested. The test concentrations were high ranging from 100 to 500 ppm. A	Java medaka adults were cultured in the laboratory and the fertilized eggs of the F2 generation were exposed to different concentrations of Roundup formulation (100, 200, 300, 400 and 500 ppm) until they hatched. The

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				regulatory endpoint is not available. There is no verification of dose levels, and the study does not conform to any guidelines nor GLP.	survival and hatching rates of the embryos, changes in the heart rate and morphological impairments were recorded. 50% of the embryos exposed to 100 ppm glyphosate died after 16 days of exposure. Several developmental abnormalities were observed in pre-hatch Java medaka embryos when exposed to different concentrations of glyphosate. No NOEC could be derived. Results only show that high concentrations of Roundup induce developmental toxicity in Java medaka but such concentrations are far above those expected in realistic conditions. The potential presence of surfactant that may influence the results has not been mentioned in the study. The study is less relevant but supplementary. The results are not reliable for regulatory risk assessment purpose.
Zabaloy M. C. et al.	2016	Soil ecotoxicity assessment of glyphosate use under field conditions: microbial activity and community structure of Eubacteria and ammonia-oxidising bacteria.	Pest management science (2016), Vol. 72, No. 4, pp. 684-91	Findings are not relateable to an EU level ecotoxicology risk assessment for Annex I renewal purposes.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zabaloy M. C. et al. 2016
Zabotkina E. A. et al.	2016	The changes of the immunocompetent cells ultrastructure in the kidney, spleen and liver in Amur	Trudy VNIRO (2016), Vol. 162, pp. 73-81	Article cannot be related to an EU level ecotoxicology risk assessment, as exposure levels cannot be confirmed and there are no end-points presented that could be used in an ecotoxicology	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		sleeper Perccottus glenii at the influence of pesticide Roundup.		assessment. The paper describes sub-lethal effects /morphological changes in the structure of mitochondria.	
Zain N. M. M. et al.	2013	Growth-inhibitory Effects of Herbicides on Soil Bacterial Population in Oil Palm Plantation	Journal of Pure and Applied Microbiology (2013), Vol. 7, No. 3, pp. 1799-1808	Paper describes mixture toxicity on soil communities of four pesticides, therefore not relevant to single active substance formulation for EU renewal. onto Annex i	The RMS agrees with the applicant's reasoning and conclusion.
Zaller J. G. et al.	2018	Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice.	Environmental science and pollution research international (2018), Vol. 25, No. 23, pp. 23215-23226	Study conducted using a formulation that is not the representative formulation for the Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zaller J. G. et al. 2018
Zaller J. G. et al.	2014	Glyphosate herbicide affects belowground interactions between earthworms and symbiotic mycorrhizal fungi in a model ecosystem.	Scientific reports (2014), Vol. 4, pp. 5634	Formulation used was Roundup which contains POEA, the latter that is not relevant in the EU.	The presence of POEA is not clearly mentioned in the paper. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zaller J. G. et al. 2014
Zanuncio C. J. et al.	2018	Glyphosate-based herbicides toxicity on life history parameters of zoophytophagous *Podisus nigrispinus* (Heteroptera: Pentatomidae)	Ecotoxicology and environmental safety (2018), Vol. 147, pp. 245-250	Based on an exposure situation where soldier bugs ere exposed on glyphosate resistant crops, which are not relevant to the EU exposure situation.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information even with glyphosate resistant crops. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zanuncio C. J. et al. 2018
Zantedeschi R. et al.	2018	Selectivity of pesticides registered for soybean crop on Telenomus podisi and Trissolcus basalis.	Pesquisa Agropecuaria Tropical (2018), Vol. 48, No. 1, pp. 52-58	Test substance identity and level of exposure cannot be confirmed by the details presented in the paper.	The RMS agrees with the applicant's reasoning and conclusion.
Zebral Y. D. et al.	2018	A glyphosate-based herbicide reduces fertility, embryonic upper thermal tolerance and	Chemosphere (2018), Vol. 196, pp. 260-269	The paper relates to the product Transorb R and not the representative formulation for the Annex I renewal. The presented data are not considered	Roundup Transorb R®was used. Surfactants were not stated in this study.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		alters embryonic diapause of the threatened annual fish Austrolebias nigrofasciatus.		relevant for use in risk assessment as they are single rates and not derived end-points.	It is very likely that the tested formulation included the surfactant polyethoxylated tallow amine (POEA) as in an other study Rocha T. L. et al., 2015, (also conducted in Brazil) "Transorb" was used. In this study it was clearly stated that Transorb contains POEA. Since August 2016, POEA is not authorized in plant protection products containing glyphosate (European Commission). Therefore it is not possible to discriminate between glyphosate and POEA. The study is considered not relevant by RMS.
Zhang M. et al.	2018	Effects of nitrification inhibitor and herbicides on nitrification, nitrite and nitrate consumptions and nitrous oxide emission in an Australian sugarcane soil	Biology and fertility of soils (2018), Vol. 54, No. 6, pp. 697-706	The paper describes the influence of a nitrification inhibitor on soil functional process when exposed to herbicides. This is a comparative assessment study that is difflcut to relate to an Annex I ecotoxicology risk assessment.	This study may provide relevant information for the risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zhang M. et al. 2018
Zhang Q. et al.	2011	An evaluation on acute toxicity of 29 pesticides to Bombyx mori	Canye Kexue (2011), Vol. 37, No. 2, pp. 343	5.4.1 case b) Relevant but supplementary information: Effects of glyphosate (95% TC) on silkworms by using the leaf dipping method: 5 g mulberry leaves were evenly immersed in 10 mL test solution for 10s. However, no useful concentration can be derived. No control results available.	The RMS agrees with the applicant's reasoning and conclusion.
Zhang Q. et al.	2016	Effects of glyphosate at environmentally relevant concentrations on the growth of and microcystin production by Microcystis aeruginosa.	Environmental monitoring and assessment (2016), Vol. 188, No. 11, pp. 632	This study presents cellular and molecular findings that are not relatable to the EU level ecotoxicology risk assessment for Annex I renewal.	Relevent parameters such as celle density were also investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zhang Q. et al. 2016

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Zhang Q. et al.	2015	Effects of glyphosate on Microcystis aeruginosa growth and related mechanisms	Anhui Nongye Kexue (2015), Vol. 43, No. 36, pp. 157-159	There are no end-points presented that could be used in an EU level ecotoxicology risk assessment.	Relevent parameters were also investigated. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zhang Q. et al. 2015
Zhang Z. et al.	2016	Acute toxicity and risk assessment of paraquat aqueous solution and its 9 alternative products to Bombyx mori	Canye Kexue (2016), Vol. 42, No. 3, pp. 483-487	The dipping technique for leaf exposure is not a recognized EU approach for toxicity testing. Not relevant to an EU level Annex I ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Zhao J. et al.	2013	Non-target effects of herbicides on soil nematode assemblages	Pest Management Science (2013), Vol. 69, No. 6, pp. 679-684	Paper discusses a soil nematode meta-analysis with no supported data presented. The resulting analysis cannot be related to an EU level Annex I risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Zhelezova A. D. et al.	2018	Structural and functional characteristics of prokaryotic complex of sod-podzolic soil influenced by herbicide glyphosate	Vestnik Moskovskogo Universiteta Seriya 17 Pochvovedenie (2018), No. 2, pp. 48-54	This is a medium to long term soil bacteria monitoring study. There are no quantifiable end-points presented nor exposure levels defined that can be related to an EU level ecotoxicology risk assessment for renewal purposes. Despite glyphosate being mentioned in the title / abstract, there is no information about glyphosate (rates used / source / purity etc.) in the paper.	The RMS agrees with the applicant's reasoning and conclusion.
Zhong G. et al.	2018	Responses of Hydrilla verticillata (L f.) Royle and Vallisneria natans (Lour.) hara to glyphosate exposure	Chemosphere (2018), Vol. 193, pp. 385-393	The paper describes enzymatic levels in aquatic plants that cannot be related to an EU level risk assessment for EU renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Zhou C. et al.	2014	Inhibition effect of glyphosate on the acute and subacute toxicity of cadmium to earthworm Eisenia fetida.	Environmental toxicology and chemistry (2014), Vol. 33, No. 10, pp. 2351-7	This paper looked at the impact of GBH in combination with cadmium in soil on earthworm toxicity. This study was a mixture assessment and thus not considered relevant for the Annex I renewal of a single a.i. containing the representative formulation for Annex I renewal.	The RMS agrees with the applicant's reasoning and conclusion.
Zhou C. et al.	2012	Does glyphosate impact on Cu uptake by, and toxicity to, the earthworm Eisenia fetida?.	Ecotoxicology (2012), Vol. 21, No. 8, pp. 2297-305	This paper looked at the impact of GBH in combination with copper in soil on earthworm toxicity. This study was a mixture assessment and thus not considered relevant for the Annex I	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				renewal of a single a.i. containing the representative formulation for Annex I renewal.	
Zhu X. et al.	2016	Herbicides interfere with antigrazer defenses in Scenedesmus obliquus.	Chemosphere (2016), Vol. 162, pp. 243-51	This paper discusses sub-lethal impacts of glyphosate on colonising activity of scenedesmus. The end-points are not releatable to the EU level ecotoxicology risk assessment for Annex I renewal.	The RMS does not agree with the applicant's reasoning and conclusion. This study may provide relevant information for the indirect effects/biodiversity assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Zhu X. et al. 2016
Zhu Y. C. et al.	2017	Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (Apis mellifera).	PloS one (2017), Vol. 12, No. 6, pp. e0178421	This study summarises that there were no effects on bees from glyphosate exposure alone. When mixed with other pesticides, effects observed, but as this is based on mixtures, it is not relevant to EU level ecotoxicology risk assessment for single active containing formulation for Annex I renewal in the EU.	The RMS agrees with the applicant's reasoning and conclusion.

Table B.9.11.1.4-2b.: From Literature Review Report KCA 9-002 (October 2020)

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
Al-Kawaz J. M.	2019	Effect of acute toxicity of glyphosate in gold fish Carassius auratus.	Annals of Tropical Medicine and Public Health, (2019) Vol. 22, No. Special Issue 5, Art No. SP173	5.4.1 case b) relevant but supplementary information: This is a dose-response laboratory study on the 96-h acute toxicity to goldfish (Carassius auratus) with an endpoint of 96-h LC50 = 14.55 ppm. 4 concentrations were tested. Behavioural, morphological and histopathological changes were recorded. No analytical verifications, no control results and no information on origin / any previous exposure of fishes is available. No statistical information provided. In addition, Glyphosate was not sufficiently documented. Fish used in test were collected from fish shops and they were not correctly reported. Previous exposure to pesticides cannot be excluded. **The article was downgraded to Category B due to its non-reliability.**	The RMS agrees with the applicant's reasoning and conclusion.
Almeida P. R. *et al.*	2019	Acute toxicity (CL50) and behavioral and morphological effects of a commercial formulation with glyphosate active ingredient in tadpoles of *Physalaemus cuvieri* (Anura, Leptodactylidae) and *Rhinella icterica* (Anura, Bufonidae).	Engenharia Sanitaria e Ambiental (2019) Vol. 24, No. 6, pp. 1115-1125	The publication is not relevant as in the conclusion, the authors indicated that the product used contains POEA. POEA is not permitted for use in formulated herbicidal products in the EU. As the performance / efficacy of herbicidal formulations is dependant on the surfactant system / co-formulants, the findings in the paper cannot be related to the representative formulation, and are therefore not relevant to the risk assessment for EU renewal. In addition, there is a lack of analytical verifications of the substance concentration in water. Unit of the endpoint is unclear and no information provided whether the endpoint refers to the formulation, glyphosate or its salt.	The RMS agrees with the applicant's reasoning and conclusion.
Erhunmwunse N. O. *et al.*	2018	Acute toxicity of glyphosate-based Isopropylamine formulation to juvenile African catfish (Clarias gariepinus).	Nigerian Journal of Basic and Applied Sciences (2018) Vol. 26, No. 2, pp. 97-101	5.4.1 case b) relevant but supplementary information: This is a dose-response laboratory study on the 96-h acute toxicity to African catfish (Clarias gariepinus) juveniles with an endpoint of 96-h LC50 = 300 mg/L. However, there is a lack of analytical verifications of the substance concentration in water. No clear origin of the fishes. Unit of the endpoint is unclear (no information whether the endpoint refers to the formulation, glyphosate or its salt). Test item cannot be identified from the article. Test design stated as being based on total residual chlorine in abstract - but it does state in the methods that OECD (1992) procedure was used, which refers	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				to the OECD 203 acute test guideline from July 1992. Concerning fish loading - if the test employed 60 L aquariums - which cannot be confirmed, the loading is too high (approx. 18 g fish/L) compared to OECD 1992 procedure for acute fish testing of approx 1 g fish/L. **The article was downgraded to Category B due to its non-reliability.**	
Fathi M. A. *et al.*	2020	Disruption of cytochrome P450 enzymes in the liver and small intestine in chicken embryos in ovo exposed to glyphosate.	Environmental science and pollution research international, (2020) Vol. 27, No. 14, pp. 16865-16875	The publication investigates the effects on antioxidant enzyme activity, histomorphology on the liver and small intestine. Enzyme, cellular and molecular level endpoints are not relevant to EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Gonzalez D. et al.	2019	Freshwater periphyton response to technical-grade and two commercial formulations of glyphosate.	Ecologia Austral, (2019) Vol. 29, No. 1, pp. 20-27	5.4.1 case b) relevant but supplementary information: The effects of a single glyphosate concentration (3 mg/L; 2, 5 and 9 days after application) provided by different means (pure glyphosate and 2 different formulations) on the structure of the microbial community in a freshwater microcosm were investigated. Pigments concentration, dry weight, ash-free dry weight, and algal density were determined. Effects on the control were provided and analytical verifications were made. An increase of Cyanobacteria and a decrease of algae abundances were registered in all treatments with the herbicide. The effect was greater for the formulations and lower with technical-grade glyphosate, suggesting that additives in the commercial formulations may enhance glyphosate effects. The test is not performed according to any OECD guidance, and no endpoints are given. The study is well written and published in an SCI journal. The article presents results for a microcosm type experiment where by 2 Litre treatment units were established with periphyton grown on substrates from a mesocosm. All substrates were pre-exposed to mesocosm water for 36 days, after which time substrates became colonised. Microbial communities were not assessed. Test water was prepared from centrifuged mesocosm waterand then 5 pre-grown substrate boards were suspecned in each of the three treatments + control. Ther test does not follow a recognised test design and there is some uncertainities with the methods used for identifying species	This study investigated the effects of pure glyphosate and 2 different formulations on the structure of the microbial community in a freshwater microcosm. Pigments concentration, dry weight, ash-free dry weight, and algal density were determined. Potentially relevant for the biodiversity/indirect effects assessment. Data gap : provide the study together with a study summary including detailed assessment of relevance and reliability.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				and for example, how were dead diatyoms deteremined. Despite these substrates being naturally colonised, there is no discussion over the zooplankton community that would also have been present on the substrates including / but not limited to rotifers. The influence of other factors on the periphyton assemablages on the substrates is not discussed.	
Guo L. *et al.*	2020	Effects of glyphosate and paraquat on root morphology and aboveground growth of Prunus persica seedlings.	Ying yong sheng tai xue bao = The journal of applied ecology, (2020) Vol. 31, No. 2, pp. 524-532	.4.1 case b) relevant but supplementary information: The aim of the study is to examine the effects of two herbicides (glyphosate and paraquat) on vegetative growth, root structure, root-tip cell mitosis and photosynthesis in peach (Prunus persica) seedlings. The growth of both shoots and roots of the P. persica seedlings was significantly inhibited by glyphosate applied at 2.5 g/L with a plant height decrease of 31. 5% compared to control. Total root length, root surface area, root volume and the number of root tips also decreased compared to the control by 39.5%, 39.5%, 49.8% and 44.6%, respectively. The test does not follow any of the recommended OECD protocols for testing terrestrial plants at the EU level. Furthermore, the test substance is not clearly identified - it is unclear whether it is a product or a technical substance. However, the test item used cannot be related to the EU representative formulation asi it was stated that the product used was a 300 g/L formulation - but no other formulation details are presented. Exposure concentrations in the spray were not confirmed. No endpoints nor apical measurements presented that can be related to an EU level risk assessment for renewal purposes. The article is not reliable.	This study investigated the effects of glyphosate (test item not clearly defined) on vegetative growth, root structure, root-tip cell mitosis and photosynthesis in peach (Prunus persica) seedlings. Potentially relevant for biodiversity assessment as little is known about sensitivity of woody species to glyphosate. Data gap : Provide a study summary including detailed assessment of relevance and reliability.
Faita M. R. *et al.*	2020	Glyphosate-based herbicides and Nosema sp. microsporidia reduce honey bee (Apis mellifera L.) survivability under laboratory conditions	Journal of Apicultural Research, (2020) Vol. 59, pp. 332-342	5.4.1 case b) relevant but supplementary information: This is an acute oral toxicity test on bees performed according to the OECD 213. Collected bees in winter and spring were orally exposed to Roundup alone, Nosema spp. spores and a combination of both. 48-h survival after exposure to Glyphosate only (calculated as 0.08 µg a.s./bee, considering an average food consumption of 30 µL/bee) was above 95% for both winter and spring collected bees. Mortality increased when exposed to the mixture with Nosema spp. spores. One single glyphosate concentration and a control was tested. The	This study investigated the mortality and food consumption of *Apis mellifera* workers infected, or not, with *Nosema microsporidia* spores and exposed to a diet containing Roundup. It is hypothesized that exposure to glyphosate

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				study is published in a SCI peer-reviewed journal and provides relevant toxicological information on the acute oral risk to bees but no endpoints are given. There are some lacking information: no RF test conducted, no positive control was used, performance of hive was not reported. In addition, RNA profiling not used as an endpoint is EU Annex I renewal ecotox risk assessment and the outcome of the study is not very useful for the risk assessment because the tested rate is sub-lethal	may lead to a greater susceptibility to pathogen infections. On this aspect, the study is considered not directly relevant for the risk assessment but still relevant for the investigation of "other types of effects" not currently covered by the risk assessment scheme. Potentially relevant for indirect effects assessment. Data gap : Provide a study summary including detailed assessment of relevance and reliability.
Kalai K. *et al.*	2019	Effect of induced chronic glyphosate toxicity in liver and kidneys of kuroiler birds.	Indian Journal of Veterinary Pathology (2019) Vol. 43, No. 3, pp. 211-216	The publication investigates biochemical, histopathological and cellular ultrastructural parameters of blood and liver tissues and only findings on cellular/molecular level are reported. Enzyme, cellular and molecular level endpoints are not relevant to EU level ecotoxicology risk assessment.	The RMS agrees with the applicant's reasoning and conclusion.
Kharat T. L. *et al.*	2016	Effect of glyphosate roundup on oxygen consumption in freshwater fish Rasbora daniconius	Ecoscan, (2016) Vol. 9, No., Spec.Iss., pp. 567-571	5.4.1 case b) relevant but supplementary information: This is a dose-response laboratory study on the 96-h acute toxicity to a local fish species (Rasbora daniconius) with an endpoint of 96-h LC50 = 5.66 mg/L. 7 concentrations were tested. Oxygen consumption was measured in a separate test when fishes are exposed to control, lethal and sub-thel concentration of the formulation in water. Behavioural and morphological observations were also made. There is a lack of analytical verifications. No statistical analysis. Glyphosate was not sufficiently documented, No information given about the control. Other relevant methodological information not provided. Wild-caught fish used in test, previous exposure to pesticides cannot be excluded. No test guideline stated. Fitness of test population unknown. Exposure test conditions, test media preparation, environmental controls - all were not	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				defined / no water quality data reported in the results. Fish weights reported, but as test design not presented, the fish loading and influence on outcome of results cannot be determined. Uncertainity in the results based on errors in the results table i.e. 70% mortality stated to have occurred at the 4.6 mg/L rate, when the text and the report table suggest only 30% mortality. **The article was downgraded to Category B due to its non-reliability.**	
Lu T. *et al.*	2020	Understanding the influence of glyphosate on the structure and function of freshwater microbial community in a microcosm.	Environmental pollution, (2020) Vol. 260, Art. No. 114012	5.4.1 case b) relevant but supplementary information: The effects of single glyphosate concentration (2.5 mg/L, 15 days) on the structure and function of microbial communities in a freshwater microcosm were investigated. This treatment did not significantly alter the physical and chemical condition of the microcosm or the composition of the main species in the community, but the transcriptions of some cyanobacteria were significantly influenced. Under glyphosate stress, the microbial community structure did not change much, but the microbes' function varied a lot. The test is not preformed according to any OECD guidance, but the outcomes on the aquatic microbial community (algae, cyanobacteria) can be relevant for the risk assessment but no endpoints are given. There is a lack of analytical verifications of the product concentration on the artificial medium and that the samples are from a lake and therefore, previous exposure to pesticides cannot be excluded.	This study investigated the effects of glyphosate (2.5 mg/L, 15 days) on the structure and function of microbial communities in a freshwater microcosm. Potentially relevant for the biodiversity/indirect effects assessment. Data gap: Provide a study summary including detailed assessment of relevance and reliability
Mestre A. P. *et al.*	2020	Effects of glyphosate, cypermethrin, and chlorpyrifos on hematological parameters of the tegu lizard (*Salvator merianae*) in different embryo stages.	Chemosphere, (2020) Vol. 252, Art. No. 126433	The publication investigates haematological parameters on reptiles' embryos and only findings at the cellular/molecular level are reported. Enzyme, cellular and molecular level endpoints are not relevant to EU level ecotoxicology risk assessment.	The publication investigates haematological parameters on reptiles' embryos and cellular/molecular level. Exposure via direct overspray to eggs at different developmental stages. Potentially relevant for the ED assessment.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Study summary of the article was proposed as a data gap.
Mottier A. *et al.*	2020	In vitro effects of glyphosate-based herbicides and related adjuvants on primary culture of hemocytes from Haliotis tuberculata.	Fish & shellfish immunology, (2020) Vol. 100, pp. 1-8	The publication investigates in vitro effects on hemocytes and only findings on cellular/molecular level are reported. Enzyme, cellular and molecular level endpoints are not relevant to EU level ecotoxicology risk assessment.	Only effects on molecular basis with no correlation seen to effect at individual level. Agree.
Nagai T.	2019	Sensitivity differences among seven algal species to 12 herbicides with various modes of action.	Journal of Pesticide Science (2019) Vol. 44, No. 4, pp. 225-232	5.4.1 case b) relevant but supplementary information: For glyphosate no data presented that could impact the endpoints used in the risk assessment as they have been achieved using a method that is not recognised at the EU level. Reference to available data is considered a secondary source and therefore not relevant to EU renewal. Validity criteria not reported. ErC50 were calculated at 96h instead of at 72h. The initial green algae biomass concentration was not reported. The test substance was not clearly identified (purity unclear). Control results are missing.	The RMS agrees with the applicant's reasoning and conclusion.
Odetti L. M. *et al.*	2020	Genotoxicity and oxidative stress in *Caiman latirostris* hatchlings exposed to pesticide formulations and their mixtures during incubation period.	Ecotoxicology and environmental safety, (2020) Vol. 193, Art. No. 110312	In vivo study on the effects of Glyphosate-based herbicides on eggs of Caiman latirostris. The formulation tested (GLY Roundup® Full II (66.2%), water-soluble herbicide (12000 mg/L), containing potassium salt) is not the EU representative formulation for the glyphosate EU renewal and therefore not relevant for the EU risk assessment.	The study investigates the effects of a glyphosate-based herbicide (GLY Roundup® Full), on eggs of *Caiman latirostris*, exposed via spray. There is not sufficient information in the study report to assess whether the tested formulation is comparable to the representative formulation. Study summary of the article was proposed as a data gap.
Pontes J. P. *et al.*	2020	A glyphosate-based herbicide in a free-choice test on parasitism, emergence, and	Journal of Plant Diseases and Protection, (2020) Vol. 127, No. 1, pp. 73-79	Roundup Original DI® (a mixture of IPA and diammounium salts) is not the EU representative formulation, therefore the article is not relevant for the glyphosate EU renewal.	The RMS does not agree with the applicant's reasoning and conclusion. The difference in

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
		female-biased sex ratio of 10 Trichogrammatidae.			formulation is not in itself a reason to consider a study as non-relevant. Data gap: Provide a study summary and a detailed assessment of reliability for the paper of Pontes J. P. et al. 2020
Ruuskanen S. *et al.*	2020	Effects of parental exposure to glyphosate-based herbicides on embryonic development and oxidative status: a long-term experiment in a bird model.	Scientific reports, (2020) Vol. 10, No. 1, pp. 6349	Roundup Flex is a potassium salt based formulation and has a different surfactant system compared to the EU representative formulation. Therefore, this publication is not relevant for the EU glyphosate renewal.	The study investigates the effects of dietary exposure to a glyphosate formulation (Roundup Flex) on the embryo development and brain biomarkers of Japanese quails. This study was proposed as a data gap.
Sanudi F. *et al.*	2018	Lethal toxicity of glyphosate herbicide on koi carp, cyprinus carpio (Linnaeus, 1758) fingerlings.	Toxicology International, (2018) Vol. 25, No. 2, pp. 139-141	5.4.1 case b) relevant but supplementary information: Bioassay experiments were conducted to determine the lethal toxicity of glyphosate herbicide on Koi carp, Cyprinus carpio fingerlings. The fishes were exposed to different concentrations of glyphosate and mortality was recorded after every 6 h for a period of 96 h. The 96 h LC50 concentration for glyphosate on Koi carp fingerlings was found to be 33.2 mg/L. There is no test item information, nor biological observation data presented to corroborate the findings, in addition no chemical analysis and therefore exposure cannot be confirmed. **The article was downgraded to Category B due to its non-reliability.**	The RMS agrees with the applicant's reasoning and conclusion.
Shitha C. *et al.*	2017	Impact of glyphosate and chlorpyriphos on chemical and biological properties of a lateritic soil	Pesticide Research Journal (2017) Vol. 29, pp. 68-74	Roundup SL is commercialized in India and it is not the EU representative formulation thus this article is not relevant for the EU glyphosate renewal. In addition, the study design, the test system and the species tested are not relevant for the European regulatory purposes. The tested soil (even for lab tests) is a local one in India. The tested species (Perionyx	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				excavatus in the case of earthworms) are also native. In addition, the field experiments are not dealing with EU representative conditions. No experiment was performed according to any of the EU recommended testing guidances/designs. No useful endpoint can be derived.	
Solis-Gonzalez G. *et al*	2019	Acute toxicity of N-(phosphonomethyl) glycine herbicide on planktonic microorganisms Artemia franciscana and Microcystis aeruginosa.	TIP Revista Especializada en Ciencias Quimico-Biologicas, (2019) Vol. 22, pp. 1-8	5.4.1 case b) relevant but supplementary information: The aim of this research was to evaluate the median lethal concentration at 24h in Artemia franciscana, as well as the median population inhibitory concentration and the coefficient of form in the cyanobacterium Microcystis aeruginosa in aquatic ecosystems. The calculated endpoint for A. franciscana was 24-h LC50 = 0.31 mg/L and for M. aeruginosa was 72-h ErC50 = 53.95 mg/L. Lack of analytical verifications during the test. Tested concentrations and dissolved oxygen (for invertebrate species) was not reported. For the additional aquatic invertebrate species, mortality was calculated at 24h (instead of at 48h). As raw data are not provided, it is not possible to check the validity criteria of the tests. The endpoints and the performance of the controls cannot be validated. **The article was downgraded to Category B due to its non-reliability.**	This study may provide data relevant for a Weight of evidence risk assessment. Data gap: Provide a study summary and a detailed assessment of reliability for the paper Solis-Gonzalez G. et al. 2019
Selvarani A. J. *et al.*	2019	Acute toxicity of glyphosate herbicide on Nile tilapia (Oreochromis niloticus)	International Journal of Current Microbiology and Applied Sciences, (2019) Vol. 8, No. 10, pp. 61-68	5.4.1 case b) relevant but supplementary information: This test was performed in a static renewal regime with Nile tilapia (Oreochromis niloticus) exposed to 5 different concentrations of glyphosate (15.33, 30.67, 61.34, 122.68 and 245.36 mg/L) for 96 hours. Mortality was recorded but also the gill, liver and kidney tissues were dissected out. Lack of analytical verifications of the substance concentration in water but exposure medium was changed every 24 h to maintain the desired concentrations. The test item is not identified. There is no chemical analysis. Water quality measurements have / appear to have only been done at the test start. A table is presented, but whether this is starting or duration derived values is unknown. Fish loading during the 96 hr test is excessive. 10 x 100 g fish in 50 litres = 20 g fish / litre. US EPA requires 0.8 g fish/L; OECD requires 1.0 g/L. This study would be considered invalid in the EU and the US for these	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				reasons. **The article was downgraded to Category B due to its non-reliability.**	
Vazquez D. E. *et al.*	2020	Chronic exposure to glyphosate induces transcriptional changes in honey bee larva: A toxicogenomic study.	Environmental pollution, (2020) Vol. 261, Art. No. 114148		This study investigated the effects of chronic exposure of a concentration of pure glyphosate on honey bee larvae regarding their gene expression profile using a transcriptomic approach. The results suggest an increase of the catabolism and oxidative metabolism in honey bee asymptomatic larvae chronically exposed to glyphosate. A maladaptive physiological response in early stages in life cycle could lead to long-term negative effects on bee populations. Potentially relevant for the investigation of "other types of effects". Data gap: Provide a study summary including detailed assessment of relevance and reliability
Villar S. *et al.*	2019	Measurement of genetic damage in Apis mellifera caused by agrochemicals using comet assay.	Current Topics in Toxicology, (2019) Vol. 15, pp. 133-139	The publication investigates the effects of acute exposure of a glyphosate formulation on adult honey bee DNA. Only findings at the cellular/molecular level are reported. Enzyme, cellular and molecular level endpoints are not relevant to EU level ecotoxicology risk assessment.The experiment was not performed according to any of the EU recommended testing guidances/designs.	This study investigated the effects of acute exposure of a glyphosate formulation on adult honey bee DNA. Potentially relevant for the investigation of "other types of effects".

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
					Data gap: Provide a study summary including detailed assessment of relevance and reliability of the paper of Villar S. et al. 2019 (Measurement of genetic damage in Apis mellifera caused by agrochemicals using comet assay.)
Ye J. et al.	2019	The Growth, Apoptosis and Oxidative Stress in Microcystis viridis Exposed to Glyphosate	Bulletin of environmental contamination and toxicology (2019) Vol. 103, No. 4, pp. 585-589	5.4.1 case b) relevant but supplementary information: Provides information on the effects of glyphosate on the growth of Microcystis viridis at 4 different concentrations every 24 h for 10 days but no endpoints are given. The algal growth inhibition test was conducted according to the OECD guideline 201-Freshwater Alga and Cyanobacteria (2011). However, as no raw data and only results in figures were presented, it is not possible to check its validity criteria. No reference substance has been tested. Analytical verifications were performed but it is not clear in the study whether they are only made at the test start or also during the study. Analytically, over a 3 day period, glyphosate is very stable under illuminated conditions. Under 240 hours exposure, it is highly unlikely that the authors could have achieved such high recoveries, hence the thought would be that the measured values presented were initial measured concentrations. The duration of the study is longer than recommended (10 days instead of 3), but growth rate is recorded after 72 h. There is not sufficient information presented to corroborate the findings.	See previous table. Data gap : Provide a study summary including detailed assessment of relevance and reliability of the paper of Ye J. et al. 2019 (The Growth, Apoptosis and Oxidative Stress in Microcystis viridis Exposed to Glyphosate)
Zhang Y. et al.	2016	Inhibitory activity of 26 herbicides against the growth of Scenedesmus obliquus	Anhui Nongye Kexue, (2016) Vol. 44, No. 23, pp. 132-133	5.4.1 case b) relevant but supplementary information: The aim of this research was to determine the inhibitory activities of 26 herbicides against the growth of the microalgae Scenedesmus obliquus using an absorption spectrophotometry method. Among the 26 herbicides, glyphosate was categorized as low toxic (72 h EyC50 = 73.9 mg/L) and glyphosate-isopropylammonium (72 h EyC50 = 2.21 mg/L) as moderately toxic. Methodology of the test is	The RMS agrees with the applicant's reasoning and conclusion.

Author(s)	Year	Title	Source	Reason for not including publication in dossier (based on relevance and reliability criteria) As proposed by the applicant	RMS conclusion
				poorly described and then only final conclusions are reported. The article was published in non-peer reviewed journal. Lack of analytical verifications during the test. pH not reported. Test substance is not clearly identified and tested rates are not reported. The response variable was given as yield, which may be needed to fulfil specific regulatory requirements in some EU countries. However, the data basis of the endpoint is unclear as it was also stated that inhibition concentration based on biomass was calculated. The inhibition rate was calculated using the absorption of the tested solutions and the conversion factor (cell number vs. absortion) is not known. The strain/ origin of the tested organisms is not suficiently reported. As raw data are not provided, it is not possible to check the validity criteria of the tests. The endpoints and the performance of the controls cannot be validated. **The article was downgraded to Category B due to its non-reliability.**	

Table B.9.11.1.4-3.: Relevant studies included in Assessment Report after detailed assessment of full-text documents for relevance: sorted by data requirement(s)

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 8.1.4	Bach N. C. *et al.*	2016	Effect on the growth and development and induction of abnormalities by a glyphosate commercial formulation and its active ingredient during two developmental stages of the South-American Creole frog, *Leptodactylus latrans*.	Environmental science and pollution research (2016), Vol. 23, No. 23, pp. 23959
CA 8.1.4	Fuentes L. *et al.*	2014	Role of sediments in modifying the toxicity of two Roundup formulations to six species of larval anurans.	Environmental toxicology and chemistry (2014), Vol. 33, No. 11, pp. 2616
CA 8.1.4	Lenkowski J. R. *et al.*	2010	Low concentrations of atrazine, glyphosate, 2,4-dichloro-phenoxyacetic acid, and triadimefon exposures have diverse effects on *Xenopus laevis* organ morphogenesis.	Journal of environmental sciences (2010), Vol. 22, No. 9, pp. 1305
CA 8.1.4	Turhan D. Ö *et al.*	2020	Developmental and lethal effects of glyphosate and a glyphosate-based product on *Xenopus laevis* embryos and tadpoles.	Bulletin of Environmental Contamination and Toxicology, (2020) Vol. 104, No. 2, pp. 173-179
CA 8.1.4	Williams B. K. *et al.*	2010	Larval responses of three midwestern anurans to chronic, low-dose exposures of four herbicides.	Archives of environmental contamination and toxicology (2010), Vol. 58, No. 3, pp. 819-827
CA 8.2.1	Gaur H. et al.	2019	Glyphosate induces toxicity and modulates calcium and NO signaling in zebrafish embryos.	Biochemical and biophysical research communications (2019 Vol. 513, No. 4, pp. 1070

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 8.2.1	Le Mer C. et al.	2013	Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (Gasterosteus aculeatus).	Ecotoxicology and environmental safety (2013), Vol. 89, pp. 174
CA 8.2.1	Zhang S. et al.	2017	Biological impacts of glyphosate on morphology, embryo biomechanics and larval behavior in zebrafish (Danio rerio).	Chemosphere (2017), Vol.181, pp. 270
CA 8.2.1 (CA 8.2.1/022, CA 8.2.1/023)	Syedkolaei-Gholami S. J. et al.	2013	Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (Cyprinus carpio, Linnaeus, 1758).	Journal of Veterinary Research (2013), Vol. 68, No. 3, pp. 257
CA 8.2.1 (CA 8.2.2.1/006)	Schweizer M. e tal.	2019	How glyphosate and its associated acidity affect early development in zebrafish (Danio rerio).	PeerJ (2019), Vol. 7, pp. e7094
CA 8.2.1 (KCA 8.2.1-021)	Antunes A. M. et al.	2017	Gender-specific histopathological response in guppies Poecilia reticulata exposed to glyphosate or its metabolite aminomethylphosphonic acid.	Journal of applied toxicology (2017), Vol. 37, No. 9, pp. 1098
CA 8.2.2 (RMS), CA 9 (Appl)	Lopes F. M. *et al.*	2014	Effect of glyphosate on the sperm quality of zebrafish *Danio rerio.*	Aquatic toxicology (2014), Vol. 155, pp. 322-6
CA 8.2.2, CA 8.2.3,CP 10.2.2, CP 10.2.3	Uren Webster T.M. et al.	2014	Effects of glyphosate and its formulation, roundup, on reproduction in zebrafish (Danio rerio).	Environmental science & technology (2014), Vol. 48, No. 2, pp. 1271-1279
CA 8.2.2, CA 8.2.5 (CA 8.2.5.1/008)	Levine S. L. et al.	2015	Aminomethylphosphonic acid has low chronic toxicity to Daphnia magna and Pimephales promelas.	Environmental toxicology and chemistry (2015), Vol. 34, No. 6,pp. 1382
CA 8.2.2.1 (CA 8.2.2.1/005)	de Brito Rodrigues L. et al	2019	Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms.	Mutation research (2019), Vol. 842, pp. 94
CA 8.2.2	Sulukan E. et al.	2017	An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (Daino rerio).	Chemosphere (2017), Vol. 180, pp. 77-85

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 8.2.3 (RMS), CA 9 (APPL)	Armiliato N. et al.	2014	Changes in ultrastructure and expression of steroidogenic factor-1 in ovaries of zebrafish Danio rerio exposed to glyphosate.	Journal of toxicology and environmental health. Part A (2014), Vol. 77, No. 7, pp. 405-14
CA 8.2.4	Avigliano L. et al.	2014	Effects of glyphosate on growth rate, metabolic rate and energy reserves of early juvenile crayfish, Cherax quadricarinatusM.	Bulletin of environmental contamination and toxicology (2014), Vol. 92, No. 6, pp. 631
CA 8.2.4	Demetrio P. M. et al.	2012	Effects of pesticide formulations and active ingredients on the coelenterate Hydra attenuata (Pallas, 1766).	Bulletin of environmental contamination and toxicology (2012), Vol. 88, No. 1, pp. 15
CA 8.2.4 (RMS), CA 9 (Appl)	Mugni H. *et al.*	2015	Acute toxicity of roundup to the nontarget organism *Hyalella curvispina*. Laboratory and field study	Toxicological and environmental chemistry (2014), Vol. 96, No. 7, pp. 1054-1063
CA 8.2.6	Lam C. H. et al.	2020	Toxicity of herbicides to cyanobacteria and phytoplankton species of the San Francisco Estuary and Sacramento-San Joaquin River Delta, California, USA.	Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering (2020), Vol. 5, pp. 107
CA 8.2.7. (CA 8.2.7/013)	Yanhui, T.. et al.	2015	Growth inhibition of two herbicides on Spirodela polyrrhiza	Nongyao Kexue Yu Guanli (2015), Vol. 36, pp 61
CA 8.2.8	Avigliano L. et al.	2018	Effects of Glyphosate on Somatic and Ovarian Growth in the Estuarine Crab Neohelice granulata, During the Pre-Reproductive Period	Water, air, and soil pollution (2018), Vol. 229, No. 2, pp. 44
CA 8.2.8	Mottier A. et al.	2013	Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, Crassostrea gigas.	Aquatic toxicology (2013), Vol. 128-129, pp. 67

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 8.2.8 (RMS), CA 9 (Appl)	Baker L. F. et al.	2016	The combined influence of two agricultural contaminants on natural communities of phytoplankton and zooplankton	Ecotoxicology, (2016) Vol. 25, No. 5, pp. 1021 32
CA 8.2.8 (RMS), CA 9 (Appl)	Baker L. F. *et al.*	2014	The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands.	Environmental toxicology and chemistry (2014), Vol. 33, No. 9, pp. 2076-85
CA 8.2.8 (RMS), CA 9 (Appl)	Canosa I. S. et al.	2019	Imbalances in the male reproductive function of the estuarine crab Neohelicegranulata, caused by glyphosate.	Ecotoxicology and environmental safety (2019), Vol. 182, pp. 109405
CA 8.2.8 (RMS), CA 9 (Appl)	Reno U. et al.	2014	The impact of Eskoba, a glyphosate formulation, on the freshwater plankton community.	Water environment research (2014), Vol. 86, No. 12, pp. 2294-300
CA 8.2.8 (RMS), CA 9 (Appl)	Xu Yanggui *et al.*	2017	Effects of glyphosate based herbicides on survival, development and growth of invasive snail (Pomacea canaliculata)	Aquatic Toxicology, (2017) Vol. 193, pp. 136 143
CA 8.2.8	Omran N. E. et al	2016	The endocrine disruptor effect of the herbicides atrazine and glyphosate on Biomphalaria alexandrina snails.	Toxicology and industrial health (2016), Vol. 32, No. 4, pp. 656-65
CA 8.2 (RMS), CA 9 (Appl) cited in CP B.9.14.1.2	Mudge J. F. *et al.*	2019	Wetland macrophyte community response to and recovery from direct application of glyphosate-based herbicides	Ecotoxicology and Environmental Safety, (2019) Vol. 183, Art. No. 109475

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 8.2.8/001	Daam, M.A. *et al.*	2019	Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae.	Ecotoxicology (2019), Vol. 28, pp. 707
CA 8.3.1, CP 10.3.1	Dai P. et al.	2018	The Herbicide Glyphosate Negatively Affects Midgut Bacterial Communities and Survival of Honey Bee during Larvae Reared in Vitro.	Journal of agricultural and food chemistry (2018), Vol. 66, No. 29, pp. 7786
CA 8.3.1.3, CP 10.3.1.5	Thompson H. M. et al.	2014	Evaluating exposure and potential effects on honeybee brood (Apis mellifera) development using glyphosate as an example.	Integrated environmental assessment and management (2014), Vol. 10, No. 3, pp. 463
CA 8.3.2 (RMS), CA 9 (Appl)	Mirande L. *et al.*	2010	Side-effects of glyphosate on the life parameters of Eriopis connexa (Coleoptera: Coccinelidae) in Argentina	Communications in Agricultural and Applied Biological Sciences, (2010) Vol. 75, No. 3, pp. 367 72
CA 8.3.2 (RMS), CA 9 (Appl) cited in CP B.9.14.1.4	Garcia-Ruiz E. *et al.*	2018	Weeds and ground-dwelling predators' response to two different weed management systems in glyphosate-tolerant cotton: a farm-scale study	PloS one, (2018) Vol. 13, No. 1, pp. e0191408
CA 8.4, CP 10.4.2.2	Santos M. J. G. et al.	2012	Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of Folsomia candida and Eisenia andrei.	Ecotoxicology (2012), Vol. 21, No. 8, pp. 2113
CA 8.4.1, CA 8.4.2.1, CA 8.5 (CA 8.4.1/005 also referenced under CA 8.4.2.1/005 and CA 8.5/005)	von Merey G. et al.	2016	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota	Environmental toxicology and chemistry (2016), Vol. 35, pp. 2742
CA 8.4.2	Correia F. V. et al.	2010	Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests.	Bulletin of environmental contamination and toxicology (2010), Vol. 85, No. 3, pp. 264

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 8.4.2	Rose M. T. et al.	2018	Minor effects of herbicides on microbial activity in agricultural soils are detected by N-transformation but not enzyme activity assays	European journal of soil biology (2018), Vol. 87, pp. 72
CA 8.5 (RMS), CA 9 (Appl)	Newman M. *et al*.	2016	Glyphosate effects on soil rhizosphere-associated bacterial communities	The Science of the Total Environment, (2016) Vol. 543, No. Pt A, pp. 155-60
CA 8.6.2	Rogacz D. et al.	2020	Ecotoxicological effects of new C-substituted derivatives of N-phosphonomethylglycine (glyphosate) and their preliminary evaluation towards herbicidal application in agriculture.	Ecotoxicology and environmental safety, (2020) Vol. 194, pp. 110331
CA 9	Agostini M. G. *et al.*	2020	Pesticides in the real world: The consequences of GMO-based intensive agriculture on native amphibians.	Biological Conservation (2020), Vol. 241, Article ID 108355
CA 9	Babalola O. O. *et al*	2019	Mortality, teratogenicity and growth inhibition of three glyphosate formulations using Frog Embryo Teratogenesis Assay-*Xenopus*.	Journal of applied toxicology (2019), Vol. 39, No. 9, pp. 1257-1266.
CA 9	Brodeur J. C. *et al.*	2014	Synergy between glyphosate- and cypermethrin-based pesticides during acute exposures in tadpoles of the common South American toad *Rhinella arenarum.*	Chemosphere, (2014) Vol. 112, pp. 70-6
CA 9	Edge *et al.*	2014	Variation in amphibian response to two formulations of glyphosate-based herbicides.	Environmental toxicology and chemistry (2014), Vol. 33, No. 11, pp. 2628-32
CA 9	Fuentes L. *et al.*	2011	Comparative toxicity of two glyphosate formulations (original formulation of Roundup® and Roundup WeatherMAX®) to six North American larval anurans.	Environmental toxicology and chemistry (2011), Vol. 30, No. 12, pp. 2756-61
CA 9	Jones D. K. *et al.*	2010	Roundup and amphibians: the importance of concentration, application time, and stratification.	Environmental toxicology and chemistry (2010), Vol. 29, No. 9, pp. 2016-25

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
CA 9	Jones D. K. *et al.*	2011	Competitive stress can make the herbicide Roundup® more deadly to larval amphibians.	Environmental Toxicology and Chemistry (2011), Vol. 30, No. 2, pp. 446-454
CA 9	Krynak K. L. *et al.*	2017	Rodeo™ Herbicide Negatively Affects Blanchard's Cricket Frogs (*Acris blanchardi*) Survival and Alters the Skin-Associated Bacterial Community.	Journal of Herpetology (2017), Vol. 51, No. 3, pp. 402-410
CA 9	Lajmanovich R. C. *et al.*	2011	Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase inhibitors.	Archives of environmental contamination and toxicology (2011), Vol. 60, No. 4, pp. 681-9
CA 9	Lajmanovich R. C. *et al.*	2013	Individual and mixture toxicity of commercial formulations containing glyphosate, metsulfuron-methyl, bispyribac-sodium, and picloram on *Rhinella arenarum* tadpoles.	Water Air and Soil Pollution (2013), Vol. 224, No. 3, pp. Article No.: 1404
CA 9	Lanctot C. *et al.*	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frog (*Lithobates sylvaticus*) tadpoles. II: agriculturally relevant exposures to Roundup WeatherMax® and Vision® under laboratory conditions.	Aquatic toxicology (2014), Vol. 154, pp. 291-303
CA 9	Munoz L. M. H. *et al.*	2015	Toxicity assesment of two agrochemicals, Roundup Active and Cosmo-Flux411F,21 to colombian anuran tadpoles. Original title: Evaluación de la toxicidad de dos agroquímicos, Roundup® Activo y Cosmo-Flux®411F, en renacuajos de anuros colombianos.	Acta Biologica Colombiana (2015), Vol. 20, No. 2, pp. 153-161
CA 9	Navarro-Martín L *et al.*	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (*Lithobates sylvaticus*)	Aquatic Toxicology, (2014) Vol. 154, pp. 278-90

Data requirement (indicated by the corresponding CA and CP data point number)	Author(s)	Year	Title	Source
			tadpoles. I:chronic laboratory exposures to VisionMax®.	
CA 9	Poletta G. L. *et al.*	2011	Genetic, enzymatic and developmental alterations observed in *Caiman latirostris* exposed *in ovo* to pesticide formulations and mixtures in an experiment simulating environmental exposure.	Ecotoxicology and Environmental Safety, (2011) Vol. 74, No. 4, pp. 852-9
CA 9	Relyea R. A.	2018	The interactive effects of predator stress, predation, and the herbicide Roundup.	Ecosphere, (2018) Vol. 9, pp. e02476
CA 9	Rissoli Zanelli R. *et al*.	2016	Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles.	Chemosphere, (2016) Vol. 156, pp. 37-44
CA 9	Ruuskanen S. *et al.*	2020	Female preference and adverse developmental effects of glyphosate-based herbicides on ecologically relevant traits in Japanese quails.	Environmental science & technology (2020), Vol. 54, No. 2, pp. 1128-1135
CA 9	Triana Velasquez T. M. *et al.*	2013	Lethal and Sublethal Effects of Glyphosate (Roundup® Active) to Embryos of Colombian Anurans. Original Title: Efectos letales y subletales del glifosato (Roundup® Activo) en embriones de anuros colombianos.	Acta Biologica Colombiana (2013), Vol. 18, No. 2, pp. 271-278
CA 9	Wagner N. *et al.*	2017	Effects of a commonly used glyphosate-based herbicide formulation on early developmental stages of two anuran species.	Environmental science and pollution research international (2017), Vol. 24, No. 2, pp. 1495-1508
M-CP 10.1.1 & 10.1.2	Edge *et al.*	2011	Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide.	Ecotoxicology and Environmental Safety 74 (2011) 1363-1369
M-CP 10.1.1 & 10.1.2	Edge *et al.*	2013	Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis*.	Science of The Total Environment Volume 444, 1 February 2013, Pages 145-152

Table B.9.11.1.4-4.: Relevant studies included in Assessment Report after detailed assessment of full-text documents for relevance: sorted by author(s)

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Agostini M. G. *et al.*	CA 9	2020	Pesticides in the real world: The consequences of GMO-based intensive agriculture on native amphibians.	Biological Conservation (2020), Vol. 241, Article ID 108355
Antunes A. M. et al.	CA 8.2.1 (KCA 8.2.1-021)	2017	Gender-specific histopathological response in guppies Poecilia reticulata exposed to glyphosate or its metabolite aminomethylphosphonic acid.	Journal of applied toxicology (2017), Vol. 37, No. 9, pp. 1098
Armiliato N. et al.	CA 8.2.3 (RMS), CA 9 (APPL)	2014	Changes in ultrastructure and expression of steroidogenic factor-1 in ovaries of zebrafish Danio rerio exposed to glyphosate.	Journal of toxicology and environmental health. Part A (2014), Vol. 77, No. 7, pp. 405-14
Avigliano L. et al.	CA 8.2.4	2014	Effects of glyphosate on growth rate, metabolic rate and energy reserves of early juvenile crayfish, Cherax quadricarinatusM.	Bulletin of environmental contamination and toxicology (2014), Vol. 92, No. 6, pp. 631
Avigliano L. et al.	CA 8.2.8	2018	Effects of Glyphosate on Somatic and Ovarian Growth in the Estuarine Crab Neohelice granulata, During the Pre-Reproductive Period	Water, air, and soil pollution (2018), Vol. 229, No. 2, pp. 44
Babalola O. O. *et al*	CA 9	2019	Mortality, teratogenicity and growth inhibition of three glyphosate formulations using Frog Embryo Teratogenesis Assay-*Xenopus*.	Journal of applied toxicology (2019), Vol. 39, No. 9, pp. 1257-1266.
Bach N. C. *et al.*	CA 8.1.4	2016	Effect on the growth and development and induction of abnormalities by a glyphosate commercial formulation and its active ingredient during two developmental stages of the South-American Creole frog, *Leptodactylus latrans*.	Environmental science and pollution research (2016), Vol. 23, No. 23, pp. 23959

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Baker L. F. et al.	CA 8.2.8 (RMS), CA 9 (Appl)	2016	The combined influence of two agricultural contaminants on natural communities of phytoplankton and zooplankton	Ecotoxicology, (2016) Vol. 25, No. 5, pp. 1021 32
Baker L. F. *et al.*	CA 8.2.8 (RMS), CA 9 (Appl)	2014	The direct and indirect effects of a glyphosate-based herbicide and nutrients on Chironomidae (Diptera) emerging from small wetlands.	Environmental toxicology and chemistry (2014), Vol. 33, No. 9, pp. 2076-85
Brodeur J. C. *et al.*	CA 9	2014	Synergy between glyphosate- and cypermethrin-based pesticides during acute exposures in tadpoles of the common South American toad *Rhinella arenarum.*	Chemosphere, (2014) Vol. 112, pp. 70-6
Canosa I. S. et al.	CA 8.2.8 (RMS), CA 9 (Appl)	2019	Imbalances in the male reproductive function of the estuarine crab Neohelicegranulata, caused by glyphosate.	Ecotoxicology and environmental safety (2019), Vol. 182, pp. 109405
Correia F. V. et al.	CA 8.4.2	2010	Effects of glyphosate and 2,4-D on earthworms (Eisenia foetida) in laboratory tests.	Bulletin of environmental contamination and toxicology (2010), Vol. 85, No. 3, pp. 264
Daam, M.A. *et al.*	CA 8.2.8/001	2019	Lethal toxicity of the herbicides acetochlor, ametryn, glyphosate and metribuzin to tropical frog larvae.	Ecotoxicology (2019), Vol. 28, pp. 707
Dai P. et al.	CA 8.3.1, CP 10.3.1	2018	The Herbicide Glyphosate Negatively Affects Midgut Bacterial Communities and Survival of Honey Bee during Larvae Reared in Vitro.	Journal of agricultural and food chemistry (2018), Vol. 66, No. 29, pp. 7786
de Brito Rodrigues L. et al	CA 8.2.2.1 (CA 8.2.2.1/005)	2019	Impact of the glyphosate-based commercial herbicide, its components and its metabolite AMPA on non-target aquatic organisms.	Mutation research (2019), Vol. 842, pp. 94
Demetrio P. M. et al.	CA 8.2.4	2012	Effects of pesticide formulations and active ingredients on the coelenterate Hydra attenuata (Pallas, 1766).	Bulletin of environmental contamination and toxicology (2012), Vol. 88, No. 1, pp. 15

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Edge *et al.*	M-CP 10.1.1 & 10.1.2	2011	Exposure of juvenile green frogs (*Lithobates clamitans*) in littoral enclosures to a glyphosate-based herbicide.	Ecotoxicology and Environmental Safety 74 (2011) 1363-1369
Edge *et al.*	M-CP 10.1.1 & 10.1.2	2013	Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis*.	Science of The Total Environment Volume 444, 1 February 2013, Pages 145-152
Edge *et al.*	CA 9	2014	Variation in amphibian response to two formulations of glyphosate-based herbicides.	Environmental toxicology and chemistry (2014), Vol. 33, No. 11, pp. 2628-32
Fuentes L. *et al.*	CA 9	2011	Comparative toxicity of two glyphosate formulations (original formulation of Roundup® and Roundup WeatherMAX®) to six North American larval anurans.	Environmental toxicology and chemistry (2011), Vol. 30, No. 12, pp. 2756-61
Fuentes L. *et al.*	CA 8.1.4	2014	Role of sediments in modifying the toxicity of two Roundup formulations to six species of larval anurans.	Environmental toxicology and chemistry (2014), Vol. 33, No. 11, pp. 2616
Garcia-Ruiz E. *et al*.	CA 8.3.2 (RMS), CA 9 (Appl) cited in CP B.9.14.1.4)	2018	Weeds and ground-dwelling predators' response to two different weed management systems in glyphosate-tolerant cotton: a farm-scale study	PloS one, (2018) Vol. 13, No. 1, pp. e0191408
Gaur H. et al.	CA 8.2.1	2019	Glyphosate induces toxicity and modulates calcium and NO signaling in zebrafish embryos.	Biochemical and biophysical research communications (2019 Vol. 513, No. 4, pp. 1070
Jones D. K. *et al.*	CA 9	2010	Roundup and amphibians: the importance of concentration, application time, and stratification.	Environmental toxicology and chemistry (2010), Vol. 29, No. 9, pp. 2016-25
Jones D. K. *et al.*	CA 9	2011	Competitive stress can make the herbicide Roundup® more deadly to larval amphibians.	Environmental Toxicology and Chemistry (2011), Vol. 30, No. 2, pp. 446-454

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Krynak K. L. *et al.*	CA 9	2017	Rodeo™ Herbicide Negatively Affects Blanchard's Cricket Frogs (*Acris blanchardi*) Survival and Alters the Skin-Associated Bacterial Community.	Journal of Herpetology (2017), Vol. 51, No. 3, pp. 402-410
Lajmanovich R. C. *et al.*	CA 9	2011	Toxicity of four herbicide formulations with glyphosate on *Rhinella arenarum* (Anura: Bufonidae) tadpoles: B-esterases and glutathione S-transferase inhibitors.	Archives of environmental contamination and toxicology (2011), Vol. 60, No. 4, pp. 681-9
Lajmanovich R. C. *et al.*	CA 9	2013	Individual and mixture toxicity of commercial formulations containing glyphosate, metsulfuron-methyl, bispyribac-sodium, and picloram on *Rhinella arenarum* tadpoles.	Water Air and Soil Pollution (2013), Vol. 224, No. 3, pp. Article No.: 1404
Lam C. H. et al.	CA 8.2.6	2020	Toxicity of herbicides to cyanobacteria and phytoplankton species of the San Francisco Estuary and Sacramento-San Joaquin River Delta, California, USA.	Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering (2020), Vol. 5, pp. 107
Lanctot C. *et al.*	CA 9	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frog (*Lithobates sylvaticus*) tadpoles. II: agriculturally relevant exposures to Roundup WeatherMax® and Vision® under laboratory conditions.	Aquatic toxicology (2014), Vol. 154, pp. 291-303
Le Mer C. et al.	CA 8.2.1	2013	Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the threespine stickleback (Gasterosteus aculeatus).	Ecotoxicology and environmental safety (2013), Vol. 89, pp. 174
Lenkowski J. R. *et al.*	CA 8.1.4	2010	Low concentrations of atrazine, glyphosate, 2,4-dichloro-phenoxyacetic acid, and triadimefon exposures have diverse effects on *Xenopus laevis* organ morphogenesis.	Journal of environmental sciences (2010), Vol. 22, No. 9, pp. 1305

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Levine S. L. et al.	CA 8.2.2, CA 8.2.5 (CA 8.2.5.1/008)	2015	Aminomethylphosphonic acid has low chronic toxicity to Daphnia magna and Pimephales promelas.	Environmental toxicology and chemistry (2015), Vol. 34, No. 6,pp. 1382
Lopes F. M. *et al.*	CA 8.2.2 (RMS), CA 9 (Appl)	2014	Effect of glyphosate on the sperm quality of zebrafish *Danio rerio.*	Aquatic toxicology (2014), Vol. 155, pp. 322-6
Mirande L. *et al*.	CA 8.3.2 (RMS), CA 9 (Appl)	2010	Side-effects of glyphosate on the life parameters of Eriopis connexa (Coleoptera: Coccinelidae) in Argentina	Communications in Agricultural and Applied Biological Sciences, (2010) Vol. 75, No. 3, pp. 367 72
Mottier A. et al.	CA 8.2.8	2013	Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, Crassostrea gigas.	Aquatic toxicology (2013), Vol. 128-129, pp. 67
Mudge J. F. *et al.*	CA 8.2 (RMS), CA 9 (Appl) cited in CP B.9.14.1.2	2019	Wetland macrophyte community response to and recovery from direct application of glyphosate-based herbicides	Ecotoxicology and Environmental Safety, (2019) Vol. 183, Art. No. 109475
Mugni H. *et al.*	CA 8.2.4 (RMS), CA 9 (Appl)	2015	Acute toxicity of roundup to the nontarget organism *Hyalella curvispina*. Laboratory and field study	Toxicological and environmental chemistry (2014), Vol. 96, No. 7, pp. 1054-1063
Munoz L. M. H. *et al.*	CA 9	2015	Toxicity assesment of two agrochemicals, Roundup Active and Cosmo-Flux411F,21 to colombian anuran tadpoles. Original title: Evaluación de la toxicidad de dos agroquímicos, Roundup® Activo y Cosmo-Flux®411F, en renacuajos de anuros colombianos.	Acta Biologica Colombiana (2015), Vol. 20, No. 2, pp. 153-161
Navarro-Martín L *et al.*	CA 9	2014	Effects of glyphosate-based herbicides on survival, development, growth and sex ratios of wood frogs (*Lithobates sylvaticus*) tadpoles. I:chronic laboratory exposures to VisionMax®.	Aquatic Toxicology, (2014) Vol. 154, pp. 278-90

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Newman M. *et al*.	CA 8.5 (RMS), CA 9 (Appl)	2016	Glyphosate effects on soil rhizosphere-associated bacterial communities	The Science of the Total Environment, (2016) Vol. 543, No. Pt A, pp. 155-60
Omran N. E. et al.	CA 8.2.8	2016	The endocrine disruptor effect of the herbicides atrazine and glyphosate on Biomphalaria alexandrina snails.	Toxicology and industrial health (2016), Vol. 32, No. 4, pp. 656-65
Poletta G. L. *et al*.	CA 9	2011	Genetic, enzymatic and developmental alterations observed in *Caiman latirostris* exposed *in ovo* to pesticide formulations and mixtures in an experiment simulating environmental exposure.	Ecotoxicology and Environmental Safety, (2011) Vol. 74, No. 4, pp. 852-9
Relyea R. A.	CA 9	2018	The interactive effects of predator stress, predation, and the herbicide Roundup.	Ecosphere, (2018) Vol. 9, pp. e02476
Reno U. et al.	CA 8.2.8 (RMS), CA 9 (Appl)	2014	The impact of Eskoba, a glyphosate formulation, on the freshwater plankton community.	Water environment research (2014), Vol. 86, No. 12, pp. 2294-300
Rissoli Zanelli R. *et al*.	CA 9	2016	Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles.	Chemosphere, (2016) Vol. 156, pp. 37-44
Rogacz D. et al.	CA 8.6.2	2020	Ecotoxicological effects of new C-substituted derivatives of N-phosphonomethylglycine (glyphosate) and their preliminary evaluation towards herbicidal application in agriculture.	Ecotoxicology and environmental safety, (2020) Vol. 194, pp. 110331
Rose M. T. et al.	CA 8.4.2	2018	Minor effects of herbicides on microbial activity in agricultural soils are detected by N-transformation but not enzyme activity assays	European journal of soil biology (2018), Vol. 87, pp. 72

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Ruuskanen S. *et al.*	CA 9	2020	Female preference and adverse developmental effects of glyphosate-based herbicides on ecologically relevant traits in Japanese quails.	Environmental science & technology (2020), Vol. 54, No. 2, pp. 1128-1135
Santos M. J. G. et al.	CA 8.4, CP 10.4.2.2	2012	Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of Folsomia candida and Eisenia andrei.	Ecotoxicology (2012), Vol. 21, No. 8, pp. 2113
Schweizer M. e tal.	CA 8.2.1 (CA 8.2.2.1/006)	2019	How glyphosate and its associated acidity affect early development in zebrafish (Danio rerio).	PeerJ (2019), Vol. 7, pp. e7094
Sulukan E. et al.	CA 8.2.2	2017	An approach to clarify the effect mechanism of glyphosate on body malformations during embryonic development of zebrafish (Daino rerio).	Chemosphere (2017), Vol. 180, pp. 77-85
Syedkolaei-Gholami S. J. et al.	CA 8.2.1 (CA 8.2.1/022, CA 8.2.1/023)	2013	Toxicity evaluation of Malathion, Carbaryle and Glyphosate in common carp fingerlings (Cyprinus carpio, Linnaeus, 1758).	Journal of Veterinary Research (2013), Vol. 68, No. 3, pp. 257
Thompson H. M. et al.	CA 8.3.1.3, CP 10.3.1.5	2014	Evaluating exposure and potential effects on honeybee brood (Apis mellifera) development using glyphosate as an example.	Integrated environmental assessment and management (2014), Vol. 10, No. 3, pp. 463
Triana Velasquez T. M. *et al.*	CA 9	2013	Lethal and Sublethal Effects of Glyphosate (Roundup® Active) to Embryos of Colombian Anurans. Original Title: Efectos letales y subletales del glifosato (Roundup® Activo) en	Acta Biologica Colombiana (2013), Vol. 18, No. 2, pp. 271-278

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
			embriones de anuros colombianos.	
Turhan D. Ö *et al.*	CA 8.1.4	2020	Developmental and lethal effects of glyphosate and a glyphosate-based product on *Xenopus laevis* embryos and tadpoles.	Bulletin of Environmental Contamination and Toxicology, (2020) Vol. 104, No. 2, pp. 173-179
Uren Webster T.M. et al.	CA 8.2.2, CA 8.2.3,CP 10.2.2, CP 10.2.3	2014	Effects of glyphosate and its formulation, roundup, on reproduction in zebrafish (Danio rerio).	Environmental science & technology (2014), Vol. 48, No. 2, pp. 1271-1279
von Merey G. et al.	CA 8.4.1, CA 8.4.2.1, CA 8.5 (CA 8.4.1/005 also referenced under CA 8.4.2.1/005 and CA 8.5/005)	2016	Glyphosate and aminomethylphosphonic acid chronic risk assessment for soil biota	Environmental toxicology and chemistry (2016), Vol. 35, pp. 2742
Wagner N. *et al.*	CA 9	2017	Effects of a commonly used glyphosate-based herbicide formulation on early developmental stages of two anuran species.	Environmental science and pollution research international (2017), Vol. 24, No. 2, pp. 1495-1508
Williams B. K. *et al.*	CA 8.1.4	2010	Larval responses of three midwestern anurans to chronic, low-dose exposures of four herbicides.	Archives of environmental contamination and toxicology (2010), Vol. 58, No. 3, pp. 819-827
Xu Yanggui *et al.*	CA 8.2.8 (RMS), CA 9 (Appl)	2017	Effects of glyphosate based herbicides on survival, development and growth of invasive snail (Pomacea canaliculata)	Aquatic Toxicology, (2017) Vol. 193, pp. 136 143
Yanhui, T.. et al.	CA 8.2.7. (CA 8.2.7/013)	2015	Growth inhibition of two herbicides on Spirodela polyrrhiza	Nongyao Kexue Yu Guanli (2015), Vol. 36, pp 61

Author(s)	Data requirement (indicated by the corresponding CA and CP data point number)	Year	Title	Source
Zhang S. et al.	CA 8.2.1	2017	Biological impacts of glyphosate on morphology, embryo biomechanics and larval behavior in zebrafish (Danio rerio).	Chemosphere (2017), Vol.181, pp. 270

B.9.11.2. Reference relied on

The literature data considered relied on are presented above in the tables B.9.11.1-3 (data sorted by author(s)) and B.9.11.1-4 (data sorted by annex point).

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 8.1.1.1-001	[redacted]	2003	MON 78623: An acute oral toxicity study with the Northern Bobwhite Report No.: 139-461 Document No.: [redacted]-2002-151 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 8.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.1-002	[redacted]	1997	Glyphosate acid. Acute oral toxicity (LD_{50}) to Bobwhite quail Report No.: [redacted] 400/963858 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.1.1 (OECD) Monograph 1998: - Monograph Trimesium:
KCA 8.1.1.1-003	[redacted]	1991	Glyphosate technical: Acute oral toxicity (LD_{50}) to the bobwhite quail Report No.: [redacted] 48/91266 Document No.: 68-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC, BCS	Y RAR 2017: - Monograph 1998: EG :AIIA-8 .1.1 Monograph Trimesium: -
KCA 8.1.1.1-004	[redacted]	1999	Avian Single-Dose Acute Oral Toxicity Test in Japanese Quail with the	Y	N	-	NUF	Y

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			chemical product Glifosate Técnico [redacted] Report No.: D.8.1-382/99 Document No.: - [redacted] GLP/GEP: N Published: N					RAR 2017: KIIA 8.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.1-005	[redacted]	1996	Glyphosate: Acute Oral Toxicity to Japanese Quail Report No.: 1413/4-1011 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 8.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.1-006	[redacted]	1996	Glyphosate: Acute Oral Toxicity to Mallard Duck Report No.: 1413/5-1011 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 8.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.1-007	[redacted]	1992	Glyphosate technical: Acute oral toxicity (LD_{50}) to the mallard duck Report No.: [redacted] 49/91843 Document No.: 107 GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC, BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.1.1 Monograph Trimesium: -
KCA 8.1.1.1-009	[redacted]	1991	AMPA: An Acute Oral Toxicity Study with the Northern Bobwhite Report No.: 139-277 Document No.: [redacted] 90-397 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.1.1 Monograph Trimesium: -
KCA 8.1.1.2/02	[redacted]	1997	Glyphosate acid: Dietary LC_{50} to the bobwhite quail Report No.: [redacted] 395/963857 Document No.: - Test lab GLP/GEP: Y Published: N	Y	N	-		Y Monograph 1998: EG:AIIA-8.1.1 Monograph Trimesium: -
KCA 8.1.1.2/05	[redacted]	1997	Glyphosate acid: Dietary LC_{50} to the mallard duck Report No.: ZCA 23	Y	N	-		Y Monograph 1998:

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			Document No.: - Test lab GLP/GEP: Y Published: N					EG:AIIA-8.1.1 Monograph Trimesium: -
KCA 8.1.1.3-001	[redacted]	1999	Glyphosate Acid: A reproduction study with the Northern Bobwhite (Colinus virginianus) Report No.: 123-186 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.1.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.3-002	[redacted]	2013	Letter concerning the study 'Glyphosate Acid: A Reproduction Study with the Northern Bobwhite (Colinus virginianus)', study report 123-186 Report No.: letter regarding 123-186 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.1.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.3-004	[redacted]	1999	Glyphosate Acid: A reproduction Study with the Mallard (Anas platyrhynchos) Report No.: 123-187 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.1.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.1.1.3-005	[redacted]	1978	One-Generation Reproduction Study - Mallard Duck; Glyphosate technical Report No.: 139-143 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 8.1.4 (OECD) Monograph 1998: EG:AIIA-8.1.3 Monograph Trimesium: -
KCA 8.2.1-001	[redacted]	2003	MON 78623: A 96-hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) Report No.: 139A-310C Document No.: [redacted]-2002-149 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 8.2.1-002	[redacted]	1995	Glyphosate acid: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) Report No.: AB0503/D Document No.: [redacted]5552/B [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.1-003	[redacted]	1995	The acute toxicity of glyphosate to Rainbow trout (Oncorhynchus mykiss) Report No.: 710/21 Document No.: - - GLP/GEP: Y Published: N	Y	N	-	HPQ	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2 .1 Monograph Trimesium: -
KCA 8.2.1-004	[redacted]	1993	Acute Toxicity Testing in Fish Test Article: 'Glyphosate isopropylamine salt' Report No.: 80-91-2328-03-93 Document No.: 2328-03-93 [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-005	[redacted]	1990	Glyphosate technical: 96-hour acute toxicity study (LC50) in the rainbow trout Report No.: 271631 Document No.: [redacted] Doc. No. 40 GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-006	[redacted]	1981	Acute toxicity of MON 0139 (Lot LURT 12011) [redacted]-81-072) to Rainbow trout (*Salmo gairdneri*) Report No.: 27202 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-007	[redacted]	1978	Acute Toxicity of Technical Glyphosate ([redacted]-78-165) to Rainbow Trout (*Salmo gairdneri*) Report No.: [redacted]-78-165 Document No.: - [redacted]	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			[redacted] GLP/GEP: N Published: N					Monograph Trimesium: -
KCA 8.2.1-008	[redacted]	1972	Four-Day Static Fish Toxicity Studies with CP 67573 in Rainbow Trout and Bluegills Report No.: [redacted]-72-104 Document No.: R2278 [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-009	[redacted]	1995	Glyphosate acid: Acute toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Report No.: AB0503/E Document No.: [redacted]5553/B [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.1-010	[redacted]	1991	Glyphosate Technical: 96-hour acute toxicity study (LC50) in the Bluegill sunfish Report No.: 271642 Document No.: [redacted] Doc. No. 44 GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	FMC	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-012	[redacted]	1978	Acute Toxicity of Technical Glyphosate to Bluegill Sunfish (*Lepomis macrochirus*) Report No.: [redacted]-78-123 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-013	[redacted]	2006	Glyphosate Technical: Acute Toxicity to Common Carp (*Cyprinus carpio*) Report No.: 2060/015 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.1-014	[redacted]	1973	Information not available Report No.: 95-00015 Document No.: - -	Y	N	-	LUX	Y RAR 2017: - Monograph

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			GLP/GEP: N Published:					1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-015	[redacted]	2000	Acute Toxicity of Glifosate Técnico [redacted] to Zebrafish (*Brachydanio rerio*) Report No.: [redacted]-D61.47/99 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	NUF	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.1-016	[redacted]	1993	Acute Toxicity Testing in Fish, Test Article: 'Glyphosate Isopropylamine Salt' Report No.: 80-91-2328-02-93 Document No.: 2328-02-93 [redacted] GLP/GEP: Y Published: N	Y	N	-	ADM	Y RAR 2017: - AIR2: II A 8.2.1 Monograph 1998: - Monograph Trimesium: -
KCA 8.2.1-017	[redacted]	1998	96-Hour Acute Toxicity Study in Rainbow trout with (Aminomethyl)Phosphonic Acid (Static) Report No.: 232469 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	ARY	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.1-018	Anonymous	1994	Information not available Report No.: 94-00499 Document No.: - GLP/GEP: Y Published: N	Y	N	-	LUX	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.1 Monograph Trimesium: -
KCA 8.2.1-020	[redacted]	1994	AMPA: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) Report No.: X582/A (FT83/92) Document No.: [redacted]5070/B [redacted] GLP/GEP: Y Published: N	Y	N	-	SYN	Y RAR 2017: KIIA 8.2.1 (OECD) Monograph 1998: - Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
KCA 8.2.2.1-001	[redacted]	2010	Glyphosate acid: Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions Report No.: 1005.029.321 Document No.: 1423-GLY [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 8.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.2.1-004	[redacted]	2011	AMPA (Aminomethylphosphonic acid): An early life-stage toxicity test with the fathead minnow (*Pimephales promelas*) Report No.: 139A-39A Document No.: [redacted]-2010-328 [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA 8.2.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.2.2-001	Anonymous	1975	Chronic toxicity of glyphosate to the Fathead minnow (*Pimephales promelas, Rafinesque*) Report No.: BN-75-129 Document No.: - [redacted] GLP/GEP: N Published: N	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2. 2 Monograph Trimesium: -
KCA 8.2.2.3-001	[redacted]	1989	Uptake, Depuration and Bioconcentration of 14C Glyphosate to Bluegill Sunfish (*Lepomis macrochirus*) Part I Report No.: [redacted]-9304 Document No.: R.D. No. 955 Volume 1 [redacted] GLP/GEP: Y Published: N	Y	N	-	BCS	Y RAR 2017: - AIR2: II B.9.2.1.3 Monograph 1998: - Monograph Trimesium: -
KCA 8.2.2.3-002	[redacted]	1989	Uptake, Depuration and Bioconcentration of 14C Glyphosate to Bluegill Sunfish (*Lepomis macrochirus*) Part II: Characterization and Quantitation of Glyphosate and Its Metabolites Report No.: [redacted]-9303 Document No.: R.D. No. 955 Volume 2	Y	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.3 Monograph Trimesium: -

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			[redacted] GLP/GEP: Y Published: N					
KCA 8.2.3-001	[redacted]	2012	Glyphosate: Fish Short-Term Reproduction Assay (FSTRA) with the Fathead Minnow (*Pimephales promelas*) Report No.: 707A-102A Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA-8.2.2 Monograph 1998: - Monograph Trimesium: -
KCA 8.2.3-002	[redacted]	2012	Glyphosate: Amphibian Metamorphosis Assay for the Detection of Thyroid Active Substances Report No.: 707A-103 Document No.: - [redacted] GLP/GEP: Y Published: N	Y	N	-	GTF	Y RAR 2017: KIIA-8.16.1 Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-001	[redacted]	2003	MON 78623: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*) Report No.: 139A-309 Document No.: WL-2002-150 Wildlife International Ltd. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-002	[redacted]	2000	Acute toxicity of glifosato IPA tecnico [redacted] to Daphnia magna Report No.: RF-D51.017/00 Document No.: - BIOAGRI Laboratorios Ltda. GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-003	[redacted]	2000	Acute toxicity of glifosate tecnico [redacted] to Daphnia magna Report No.: RF-D51.39/99 Document No.: - BIOAGRI Laboratorios Ltda. GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-004	[redacted]	1996	Glyphosate acid: Acute toxicity to *Daphnia magna* Report No.: AB0503/C Document No.: BL5551/B	N	N	-	SYN	Y RAR 2017: KIIA 8.3.1.1

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			Brixham Environmental Laboratory ZENECA Limited GLP/GEP: Y Published: N					(OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-005	[redacted]	1995	The akute toxicity of glyphosate to *Daphnia magna* Report No.: 710/22 Document No.: - - GLP/GEP: Y Published: N	N	N	-	HPQ	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.4 Monograph Trimesium: -
KCA 8.2.4.1-006	[redacted]	1995	Acute toxicity study in *Daphnia magna* with Glyfosaat Report No.: 141863 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.4 Monograph Trimesium: -
KCA 8.2.4.1-007	[redacted]	1994	Acute Toxicity in *Daphnia magna*; Test Article: 'Glyphosate isopropylamine salt' Report No.: 83-91-0737-00-93 Document No.: - IBR Forschungs GmbH GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-008	[redacted]	1993	Information not available Report No.: 94-00549 (typo error in the Monograph: 95-00549) Document No.: - - GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2 .4 Monograph Trimesium: -
KCA 8.2.4.1-009	[redacted]	1990	48-Hour Acute Toxicity of Glyphosate Technical to *Daphnia magna* (OECD-Immobilization Test) Report No.: 272968 Document No.: 37GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	-	FMC	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: EG:AIIA-8.2.4 Monograph

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
								Trimesium: -
KCA 8.2.4.1-010	[redacted]	1981	Acute toxicity of MON 0139 (Lot LURT 12011) (AB-81-074) to *Daphnia magna* Report No.: 27203 Document No.: AB-81-074 Analytical Bio Chemistry Laboratories Inc. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2. 3 Monograph Trimesium: -
KCA 8.2.4.1-011	[redacted]	1978	Acute Toxicity of Technical Glyphosate (AB-78-201) to *Daphnia magna* Report No.: AB 78-201 Document No.: - Analytical Bio Chemistry Laboratories Inc. GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: EG:AIIA-8.2.4 Monograph Trimesium: -
KCA 8.2.4.1-012	[redacted]	1998	Acute Toxicity Study in *Daphnia magna* with (Aminomethyl)Phosphonic Acid (Static) Report No.: 232471 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 8.3.1.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-013	[redacted]	1994	AMPA: Acute toxicity to *Daphnia magna* Report No.: X582/C Document No.: BL5061/B Brixham Environmental Laboratory ZENECA Limited GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.1-014	[redacted]	1991	Acute toxicity of AMPA to *Daphnia magna* Report No.: 38988 Document No.: AB-90-401 ABC Laboratories Inc., Environmental Biology Division GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.3 Monograph Trimesium: -
KCA 8.2.4.1-015	[redacted]	2011	HMPA (Hydroxymethylphosphonic acid): A 48-hour static acute toxicity test with the	N	N	-	GTF	Y RAR 2017: KIIA 8.3.1.1

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			cladoceran (*Daphnia magna*) Report No.: 139A-395 Document No.: WL-2010-329 Wildlife International Ltd. GLP/GEP: Y Published: N					(OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.4.2-001	[redacted]	1996	Glyphosate Acid: Acute toxicity to mysid shrimp (*Mysidopsis bahia*) Report No.: AB0503/H Document No.: BL5713/B Brixham Environmental Laboratory ZENECA Limited GLP/GEP: Y Published: N	N	N	-	GRG	N
KCA 8.2.4.2-002	[redacted]	1978	Toxicity of seven test materials to mysid shrimp *Mysidopsis bahia* Report No.: BP-78-4-032 Document No.: - EG&G, Bionomics Marine Research Laboratory GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.8 Monograph Trimesium: -
KCA 8.2.4.2-003	[redacted]	1996	Glyphosate Acid: Acute toxicity to larvae of the Pacific oyster (*Crassostrea gigas*) Report No.: AB0503/G Document No.: BL5714/B Brixham Environmental Laboratory ZENECA Limited GLP/GEP: Y Published: N	N	N	-	GRG	N
KCA 8.2.4.2-004	[redacted]	1985	Acute Toxicity of Roundup (Technical) to Atlantic Oyster (*Crassostrea virginica*) Report No.: BN-73-79 Document No.: - Bionomics, Inc. GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.4 Monograph Trimesium: -
KCA 8.2.5.1-001	[redacted]	1999	Glyphosate acid: Chronic toxicity to *Daphnia magna* Report No.: AF0497/B Document No.: BL6535/B Brixham Environmental Laboratory, ZENECA Limited	N	N	-	SYN	Y RAR 2017: KIIA 8.3.2.1 (OECD) Monograph 1998: - Monograph

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			GLP/GEP: Y Published: N					Trimesium: -
KCA 8.2.5.1-002	[redacted]	1995	*Daphnia magna*, Reproduction Test with Glyfosaat Report No.: 141874 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: - Monograph 1998: EG :AIIA-8. 2. 5 Monograph Trimesium: -
KCA 8.2.5.1-003	[redacted]	1993	21-day Reproduction Test in *Daphnia* Test Article: Glyphosate isopropylamine salt Report No.: 80-91-2328-05-93 Document No.: - IBR Forschungs GmbH GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: - Monograph 1998: EG :AIIA-8. 2. 4 Monograph Trimesium: -
KCA 8.2.5.1-004	[redacted]	1990	Influence of glyphosate on the reproduction of *Daphnia magna* Report No.: 250795 Document No.: CHA Doc.No. 24 GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	-	FMC	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.5 Monograph Trimesium: -
KCA 8.2.5.1-005	[redacted]	1989	21-Day Prolonged Static Renewal Toxicity of Glyphosate Technical to *Daphnia magna* Report No.: 37757 Document No.: AB-89-58 Analytical Bio-Chemistry Laboratories lnc., Aquatic Toxicology Division GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.5 Monograph Trimesium: -
KCA 8.2.5.1-006	[redacted]	1982	Chronic Toxicity of Glyphosate to *Daphnia magna* Under Flow-Through Test Conditions Report No.: AB-82-036 Document No.: - Analytical Biochemistry Laboratories lnc. GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2. 5 Monograph Trimesium: -
KCA 8.2.5.1-007	[redacted]	2011	AMPA (Aminomethylphosphonic acid): A semi-static life cycle toxicity test with the	N	N	-	GTF	Y RAR 2017: KIIA 8.3.2.1

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			Cladoceran (*Daphnia magna*) Report No.: 139A-393 Document No.: WL-2010-327 Wildlife International Ltd. GLP/GEP: Y Published: N					(OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.5.1-009	[redacted]	2020	Statistical evaluation (non-GLP) of the study BL6535/B on the chronic toxicity of Glyphosate acid technical to Daphnia magna under static-renewal conditions Report No.: 110054-012 Document No.: -- knoell Germany GmbH GLP/GEP: non-GLP Published: N	N	N	-	GRG	N
KCA 8.2.5.1-010	[redacted]	2020	Statistical evaluation (non-GLP) of the study 141874 on the chronic toxicity of Glyphosate to Daphnia magna under static-renewal conditions Report No.: 110054-013 Document No.: -- knoell Germany GmbH GLP/GEP: non-GLP Published: N	N	N	-	GRG	N
KCA 8.2.5.1-011	[redacted]	2020	Statistical evaluation (non-GLP) of the study 80-91-2328-05-93 on the chronic toxicity of Glyphosate Isopropylamine salt to Daphnia magna under static-renewal conditions Report No.: 110054-014 Document No.: -- knoell Germany GmbH GLP/GEP: non-GLP Published: N	N	N	-	GRG	N
KCA 8.2.5.1-012	[redacted]	2020	Statistical evaluation (non-GLP) of the study 250795 on the chronic toxicity of Glyphosate to Daphnia magna under static-renewal conditions Report No.: 110054-015 Document No.: -- knoell Germany GmbH GLP/GEP: non-GLP Published: N	N	N	-	GRG	N
KCA 8.2.5.1-013	[redacted]	2020	Statistical evaluation (non-GLP) of the study AB 82-036 on the chronic toxicity	N	N	-	GRG	N

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			of Glyphosate to Daphnia magna under flow-through conditions Report No.: 110054-016 Document No.: -- knoell Germany GmbH GLP/GEP: non-GLP Published: N					
KCA 8.2.5.1-014	[redacted]	2020	Statistical evaluation (non-GLP) of the study 139A-393 on the chronic toxicity of Aminomethylphosphonic acid to Daphnia magna under static-renewal conditions Report No.: 110054-017 Document No.: -- knoell Germany GmbH GLP/GEP: non-GLP Published: N	N	N	-	GRG	N
KCA 8.2.5.3-001	[redacted]	2020	MON 77973: A Study on the Toxicity to the Sediment Dweller *Chironomus riparius* Using Spiked Water - Interim Report Report No.: 20FV2ME Document No.: ECT-2019-0362 ECT Oekotoxikologie GmbH and CIP – Chemisches Institut Pforzheim GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 8.2.6.1-001	[redacted]	2002	A study on the Toxicity of Glyphosate isopropylamine salt 62.5% to Algae (*Pseudokirchneriella subcapitata*) Report No.: A-99-02-04 Document No.: - ECT Oekotoxikologie GmbH; and BATTELLE, Geneva Research Centres, Agrochemical Product Development GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.6.1-003	[redacted]	2000	Acute toxicity of glifosate tecnico [redacted] to *Selenastrum capricornutum* Report No.: RF-D2.44/99 Document No.: - BIOAGRI Laboratorios Ltda.	N	N	-	NUF	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph

Data Point	Author(s)	Year	Title Report No. Document No. Source (where different from company) GLP/ Officially recognised testing facilities[2,3] Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previously used[1] Y/N If yes, for which data point?
			GLP/GEP: Y Published: N					Trimesium: -
KCA 8.2.6.1-004	[redacted]	2020	Statistical evaluation (non-GLP) of the study RF-D2.44/99 on the toxicity of glifosate tecnico [redacted] to *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) under static conditions Report No.: 110054-001 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.6.1-005	[redacted]	1995	Glyphosate acid: Toxicity to the green alga *Selenastrum capricornutum* Report No.: AB0503/B Document No.: BL5550/B Brixham Environmental Laboratory, ZENECA Limited GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.6.1-006	[redacted]	2020	Statistical evaluation (non-GLP) of the study BL5550/B on the toxicity of Glyphosate acid to *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) under static conditions Report No.: 110054-002 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.6.1-007	[redacted]	1995	Fresh Water Algal Growth Inhibition Test with Glyfosaat Report No.: 141896 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2. 6 Monograph Trimesium: -
KCA 8.2.6.1-008	[redacted]	1995	Fresh water algal growth inhibition test with glyphosaat Report No.: R481 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	GTT	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.5 Monograph

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								Trimesium: -
KCA 8.2.6.1-009	[redacted]	1987	The toxicity of glyphosate technical to *Selenastrum capricornutum* Report No.: 1092-02-1100-1 Document No.: - Malcolm Pirnie Inc. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: Monograph 1998: - EG:AIIA-8. 2. 6 Monograph Trimesium: -
KCA 8.2.6.1-010	[redacted]	2020	Statistical evaluation (non-GLP) of the study 1092-02-1100-1 on the toxicity of Glyphosate Technical to *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) under static conditions Report No.: 110054-003 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.6.1-011	[redacted]	1995	Glyphosate: Algal inhibition test Report No.: 710/12 Document No.: - - GLP/GEP: Y Published: N	N	N	-	HPQ	Y RAR 2017: - Monograph 1998: EG:AIIA-8.2.6 Monograph Trimesium: -
KCA 8.2.6.1-012	[redacted]	1994	Testing of toxic effects of aminomethylphosphonic acid (AMPA) on the single cell green alga *Scenedesmus subspicatus*. Report No.: XX-93-271 Document No.: - - GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2 .6 Monograph Trimesium: -
KCA 8.2.6.1-016	[redacted]	1998	Fresh Water Algal Growth Inhibition Test with (Aminomethyl)Phosphonic Acid Report No.: 232458 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.6.1-017	[redacted]	2020	Statistical evaluation (non-GLP) of the study 232458 on the toxicity of (Aminomethyl) phosphonic	N	N	-	GRG	N

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			acid (AMPA) to *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) under static conditions Report No.: 110054-004 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N					
KCA 8.2.6.1-019	[redacted]	2011	HMPA (hydroxymethylphosphonic acid): A 72-hour toxicity test with the freshwater alga (*Pseudokirchneriella subcapitata*) Report No.: 139A-396A Document No.: WL-2010-330 Wildlife International Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.6.1-020	[redacted]	2020	Statistical evaluation (non-GLP) of the study 139A-396A on the toxicity of Hydroxymethyl phosphonic acid (HMPA) to *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*) under static conditions Report No.: 110054-005 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.6.2-001	[redacted]	1996	Glyphosate acid: Toxicity to blue-green alga *Anabaena flos-aquae* Report No.: AB0503/J Document No.: BL5698/B Brixham Environmental Laboratory, ZENECA Limited GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.6.2-002	[redacted]	1987	The toxicity of glyphosate technical to *Anabaena flos-aquae* Report No.: 1092-02-1100-4 Document No.: - Malcolm Pirnie Inc. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2. 6 Monograph

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								Trimesium: -
KCA 8.2.6.2-003	[redacted]	2020	Statistical evaluation (non-GLP) of the study 1092-02-1100-4 on the toxicity of Glyphosate technical to *Anabaena flos-aquae* under static conditions Report No.: 110054-006 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.6.2-005	[redacted]	1987	The toxicity of glyphosate technical to Navicula pelliculosa Report No.: 1092-02-1100-2 Document No.: - Malcolm Pirnie Inc. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 2. 6 Monograph Trimesium: -
KCA 8.2.6.2-006	[redacted]	1996	Glyphosate acid: Toxicity to the marine alga *Skeletonema costatum* Report No.: AB0503/I Document No.: BL5684/B Brixham Environmental Laboratory, ZENECA Limited GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 8.4 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.6.2-007	[redacted]	2020	Statistical evaluation (non-GLP) of the study BL5684/B on the toxicity of Glyphosate acid to *Skeletonema costatum* under static conditions Report No.: 110054-007 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	N	GRG	N
KCA 8.2.7-001	[redacted]	2002	IPA Salt of Glyphosate: Effects on *Lemna minor* Report No.: CEMR-1873 Document No.: - CEM Analytical Services Ltd. GLP/GEP: Y Published: N	N	N	-	SIN	Y RAR 2017: KIIA 8.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.7-002	[redacted]	2020	Statistical evaluation (non-GLP) of the study CEMS-1873 on the toxicity of Glyphosate isopropylamine	N	N	-	GRG	N

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			(IPA) salt to *Lemna minor* under static conditions Report No.: 110054-008 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N					
KCA 8.2.7-003	[redacted]	1999	Glyphosate 62% IPA-Salt, aquatic plant toxicity test using *Lemna gibba* Report No.: 980909FH Document No.: Study-No. TLA60871 Dr. U. Noack-Laboratorium für Angewandte Biologie GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: KIIA 8.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.7-004	[redacted]	2020	Statistical evaluation (non-GLP) of the study TLA60871 on the toxicity of Glyphosate 62% IPA-Salt to *Lemna gibba* under static conditions Report No.: 110054-009 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.7-005	[redacted]	1996	GLYPHOSATE ACID: Toxicity to duckweed (*Lemna gibba*) Report No.: AB0503/L Document No.: BL5662/B Brixham Environmental Laboratory, ZENECA Limited GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 8.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.7-006	[redacted]	2020	Statistical evaluation (non-GLP) of the study BL5662/B on the toxicity of Glyphosate acid to *Lemna gibba* under static conditions Report No.: 110054-010 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.7-007	[redacted]	1987	The Toxicity of Glyphosate Technical to *Lemna gibba* Report No.: 1092-02-1100-5 Document No.: - Malcolm Pirnie Inc. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG :AIIA-8. 2. 8 Monograph

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								Trimesium: -
KCA 8.2.7-008	[redacted]	2020	Statistical evaluation (non-GLP) of the study 1092-02-1100-5 on the toxicity of Glyphosate to *Lemna gibba* under static conditions Report No.: 110054-011 Document No.: - knoell Germany GmbH GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.2.7-009	[redacted]	1987	The toxicity of glyphosate technical to *Lemna gibba*. Report No.: XX-88-416 Document No.: - GLP/GEP: N Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: G:AIIA-8.2 .8 Monograph Trimesium: -
KCA 8.2.7-011	[redacted]	2012	Effect of AMPA (Aminomethylphosphonic acid) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment, with a subsequent Recovery Period Report No.: CHE-022/4-80/A Document No.: - Fraunhofer-Institute for Molecular Biology and Applied Ecology (IME) GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.2.7-012	[redacted]	2011	HMPA (hydroxymethylphosphonic acid): A 7-day static-renewal toxicity test with Duckweed (*Lemna gibba* G3) Report No.: 139A-397 Document No.: WL-2010-331 Wildlife International, Ltd. GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.6 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.1-002	[redacted]	1998	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*) Report No.: FN9700 Document No.: - National Bee Unit, Central Science Laboratory, Sand	N	N	-	SYN	Y RAR 2017: KIIA 8.7.1 (OECD) Monograph 1998: - Monograph

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			Hutton GLP/GEP: Y Published: N					Trimesium: -
KCA 8.3.1.1.1-003	[redacted]	1996	Glyphosate: Acute contact and oral toxicity to honeybees Report No.: 1413/3-1018 Document No.: - Corning Hazleton (Europe) GLP/GEP: Y Published: N	N	N	-	NUF/ FMC	Y RAR 2017: KIIA 8.7.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.1-004	[redacted]	1995	Testing Toxicity to Honeybee - *Apis mellifera L.* (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.) Report No.: 95 10 48 065 Document No.: - BioChem GmbH Karlsruhe GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: KIIA 8.7.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.1-005	[redacted]	1995	Honey Bees (*Apis mellifera L.*), oral toxicity study in the laboratory with Glyphosate Report No.: 141907 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 8.7.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.1-007	[redacted]	2017	MON 0139: Acute Oral and Contact Toxicity to the Bumble Bee, *Bombus terrestris L.* under Laboratory Conditions Report No.: S16-06634 Document No.: EPS-2016-0622 (MSL-0028880) Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 8.3.1.1.2-001	[redacted]	2003	Laboratory bioassays to determine acute oral and contact toxicity of MON 78623 to the honeybee, *Apis mellifera* Report No.: MON-02-10 Document No.: MT-2002-108 Mambo-Tox Ltd. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: KIIA 8.7.1 (OECD) Monograph 1998: - Monograph Trimesium: -

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KCA 8.3.1.1.2-002	[redacted]	2000	Acute Contact Toxicity of GLIFOSATO IPA TECHNICO [redacted] to Honey bee (*Apis mellifera L.*) Report No.: RF-D4.017/00 Document No.: - BIOAGRI Laboratorios Ltda. GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 8.7.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.2-003	[redacted]	1998	Glyphosate Acid: Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*) Report No.: FN9700 Document No.: - National Bee Unit, Central Science Laboratory, Sand Hutton GLP/GEP: Y Published: N	N	N	-	SYN	Y RAR 2017: KIIA 8.7.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.2-004	[redacted]	1996	Glyphosate: Acute contact and oral toxicity to honeybees Report No.: 1413/3-1018 Document No.: - Corning Hazleton (Europe) GLP/GEP: Y Published: N	N	N	-	NUF	Y RAR 2017: KIIA 8.7.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.2-005	[redacted]	1995	Testing Toxicity to Honeybee - *Apis mellifera L.* (laboratory) according to EPPO Guideline No 170. Glyphosate (tec.) Report No.: 95 10 48 065 Document No.: - BioChem GmbH Karlsruhe GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: KIIA 8.7.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.2-006	[redacted]	1995	Honey Bees (*Apis mellifera L.*), contact toxicity study in the laboratory with Glyphosate Report No.: 142335 Document No.: - NOTOX B.V. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 8.7.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.3.1.1.2-008	[redacted]	2017	MON 0139: Acute Oral and Contact Toxicity to the Bumble Bee, *Bombus terrestris L.* under Laboratory Conditions Report No.: S16-06634 Document No.: EPS-2016-0622 (MSL-0028880)	N	Y	First submission in EU	GRG	N

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			Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP/GEP: Y Published: N					
KCA 8.3.1.1.2-009	[redacted]	2017	MON 0139: Acute Contact Toxicity to the Solitary Bee, *Osmia bicornis* under Laboratory Conditions Report No.: S17-00083 Document No.: EPS-2016-0623 (MSL-0028894) Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 8.3.1.2-001	[redacted]	2017	MON 0139: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera L.*) in the Laboratory Report No.: 118401136 Document No.: IO-2016-0508 (MSL0029007) ibacon GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 8.3.1.3-001	[redacted]	2020	MON 0139 - Repeated exposure of honey bee larvae (*Apis mellifera L.*) under laboratory conditions Report No.: 19 48 BLC 0068 Document No.: BI-2018-0721 TRR0000053 BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	Y	First submission in EU	GRG	N
KCA 8.3.1.4-001	[redacted]	2012	Glyphosate: Evaluating potential effects on honeybee brood (*Apis mellifera*) development Report No.: V7YH1001 Document No.: - Environmental Risk Team, Food and Environmental Safety Programme, The Food and Environment Research Agency, Sand Hutton	N	N	-	GTF	Y RAR 2017: KIIA 8.7.4 (OECD) Monograph 1998: - Monograph Trimesium: -

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			GLP/GEP: Y Published: N					
KCA 8.4.1-001	[redacted]	2009	MON0139 - Sublethal toxicity to the earthworm *Eisenia fetida* Report No.: 09 10 48 056 S Document No.: - BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.4.1-002	[redacted]	2000	A laboratory investigation of the effects of Glyphosate and its breakdown product AMPA on reproduction in the earthworm *Eisenia fetida* Report No.: CEMR-1173 Document No.: CE-1999-257 CEM Analytical Services Ltd. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.4.1-003	[redacted]	2003	Laboratory determination of the side-effects of aminomethyl phosphonic acid (AMPA) on the reproductive performance of earthworms (*Eisenia fetida*) using artificial soil substrate Report No.: 01-64-077-ES Document No.: - PHYTOSAFE s.a.r.l. GLP/GEP: Y Published: N	N	N	-	ARY	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.4.1-004	[redacted]	2002	AMPA - Earthworm (*Eisenia fetida*), effects on reproduction Report No.: RRR84121 Document No.: 011120FB FSG DR.U.NOACK-LABORATORIUM FÜR ANGEWANDTE BIOLOGIE GLP/GEP: Y Published: N	N	N	-	ADM	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.4.2.1-001	[redacted]	2010	MON0139 - Effects on the reproduction of the collembolans *Folsomia candida* Report No.: 09 10 48 057 S Document No.: - BioChem agrar Labor für	N	N	-	GTF	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph

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			biologische und chemische Analytik GmbH GLP/GEP: Y Published: N					Trimesium: -
KCA 8.4.2.1-002	[redacted]	2009	MON0139 - Effects on the reproduction of the collembolans *Folsomia* Report No.: 09 10 48 058 S Document No.: - BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.4.2.1-003	[redacted]	2010	AMPA - Effects on the Reproduction of the collembolans *Folsomia candida* Report No.: 10 10 48 054 S Document No.: - BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.4.2.1-004	[redacted]	2010	AMPA - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer* Report No.: 10 10 48 053 S Document No.: - BioChem agrar Labor für biologische und chemische Analytik GmbH GLP/GEP: Y Published: N	N	N	-	GTF	Y RAR 2017: KIIA 8.9.2 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.5-001	[redacted]	2014	Glyphosate technical (MON77973): Effect on Soil Microbial Nitrogen Transformations Report No.: CEMR-6237 Document No.: - CEM Analytical Services Ltd. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: KIIA 8.10.1 (OECD) Monograph 1998: - Monograph Trimesium: -
KCA 8.5-004	[redacted]	2010	AMPA - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests) Report No.: 10 10 48 010 C/N Document No.: - BioChem agrar Labor für biologische und chemische Analytik GmbH	N	N	-	GTF	Y RAR 2017: KIIA 8.10.1 (OECD) Monograph 1998: - Monograph Trimesium: -

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			GLP/GEP: Y Published: N					
KCA 8.6.2-001	[redacted]	1994	Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study using Glyphosate Report No.: 93235 Document No.: R.D. 1219, MSL-13320 Pan-Agricultural bbs Inc. GLP/GEP: Y Published: N	N	N	-	BCS	Y RAR 2017: - Monograph 1998: EG:AIIA-8. 6 Monograph Trimesium: -
KCA 8.7-001	[redacted]	2020	Glyphosate: Indirect effects via trophic interaction - A Practical Approach to Biodiversity Assessment Report No.: TRR0000305 Document No.: - - GLP/GEP: N Published: N	N	N	-	GRG	N
KCA 8.8-002	[redacted]	1990	Assessment of the acute toxicity of glyphosate technical on aerobic waste-water bacteria Report No.: 277830 Document No.: 25 GLY RCC Umweltchemie AG GLP/GEP: Y Published: N	N	N	-	FMC	Y RAR 2017: KIIA 8.15 (OECD) Monograph 1998: - Monograph Trimesium: -

[1] In order to facilitate the compilation of the final list of the tests and studies relied upon and the corresponding data protection, indicate whether the study was used in the previous DAR/RAR or, when the information is available, whether the study was already submitted in the framework of national authorisations.
[2] See Art.3 of Annex of Regulation No 283/2013 and 284/2013
[3] The RMS shall check that the GLP statement has been properly signed in the study report, that the study results are properly reported in accordance with GLP standards and following the relevant guidance by OECD on the review of the GLP status of non-clinical safety data (currently under development).