European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Napropamide-M

Volume 3 – B.9 (AS)

Rapporteur Member State: United Kingdom

Version History

When	What
June 2017	Initial DAR

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B.9. ECOTOXICOLOGY DATA

Napropamide-M is a herbicide proposed for use in controlling broad-leaved and grass weeds in a range of annual and perennial crops. It is the resolved single isomer version of racemic napropamide which has been established on the market in plant protection products for a number of years. As such, napropamide-M is considered a new substance. The applicant has submitted studies that were submitted to support the Annex I inclusion of the the racemic mixture; these have been accepted by the RMS and have not been re-evaluated. napropamide-M is formulated as a soluable concentrate product 'D-Devrinol 450 SC' containing 450 g napropamide-M/L.

'D-Devrinol 450 SC' is to be applied pre-sowing (summer-autumn) and pre-planting/ sowing (spring-summer) at a maximum individual rate of 765 g a.s./ha for one application. Applications to winter oilseed rape and brassica vegetable crops are to be made via ground spray and incorporation.

Table B.9-1 Maximum intended use rates for napropamide-M in the product D-Devrinol 450 SC in Europe

Crop group	Application rate (g a.s./ha)	Number of applications	Application type	Application timing
Winter oilseed rape	765	1	Ground spray ± incorporation	Pre-sowing summer-autumn
Brassicas vegetable crop	765	1	Ground spray ± incorporation	Pre-planting / sowing spring-summer

Table B.9-2: Ecotoxicologically identified metabolites

Compartments of relevance	Substance
Soil	None identified
Surface water	Naphthalen-1-ol N, N-diethyl-2-(4-hydroxy-1-naphthyl) propanamide [napropamide isomer I] N, N-diethyl-2-(1-hydroxy-2-naphthyl) propanamide [napropamide isomer II]
Sediment	None identified
Ground water	None identified

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1 Acute toxicity to birds

A study was conducted with Japanese quail to determine the acute oral toxicity of napropamide-M to birds. The study has not previously been reviewed and so a study summary is provided below.

Report

[redacted] (2013) Acute oral toxicity (LD_{50}) study of napropamide-M technical in Japanese quail, *Coturnix coturnix japonica.* [redacted] Unpublished report No.: 516-3-08-6173

Guidelines

OECD 223 Avian Acute oral toxicity test.

GLP

Yes (certified laboratory)

Materials and Methods

Test material	Napropamide-M Technical
Description:	Beige colour crystalline powder.
Lot/Batch #:	UPH-08/DNE-263/Tech/20121226
Purity:	97.26% w/w (total D- + L-isomer)
Stability of test compound:	Not stated.
Reference substance:	N/A

Test animals	
Species:	Japanese quail *(coturnix coturnix japonica)*
Description:	Healthy females in mature plumage, pre breeding condition.
Source:	[redacted].

Experimental dates: 31st of May 2013 – 29th of June 2013.

Study Design

Adult Japanese quails used in the study were in mature plumage but not in breading condition and were free from any avian disease. Healthy female birds of 5 weeks of age were acclimatised to test conditions for 16 days prior to the oral administration of test item. Birds were housed individually in stainless steel wire-mesh bottomed pens (900 cm^2). Maximum and minimum temperatures were recorded daily and ranged from 22-24 ºC. The environmental conditions were controlled at 66-75 % relative humidity, light intensity of 118 to 134 Lux and an 8 hr light:16 hr darkness cycle with 15 air changes per hour. The birds were fed a layer mash diet and filtered water *ad libitum* with the exception of an overnight fasting (14.5 hrs) prior to dosing and 2 hrs post dosing.

Napropamide-M technical was not sufficiently soluble in distilled water so vegetable oil was used as the vehicle. The test solution was thoroughly stirred prior to dosing. Two control groups were used, one a vehicle control (vegetable oil) and the other distilled water. Fifteen individuals were randomly allocated into the three groups with 5 individual females per group. The dose volume used was 10.0 mL/kg body weight for each of the groups, the volume allocated to each bird was calculated based on body weight, all birds were administered a single dose via gavage using a glass syringe and a catheter.

All birds were observed for signs of toxicity, abnormal behaviour and mortality at 30 minutes, 1 and 2 hours post dosing on day 0 and subsequently once daily for 14 days. Birds were observed continuously during the first two hours for potential regurgitation. Body weight was recorded on day 0 and on day 3, 7 and at study termination on day 14. Feed consumption was recorded daily for each cage and calculated for periods of 0-3, 4-7 and 8-14 days.

As the study was conducted as a limit test and no mortality occurred at the limit dose, the LD_{50} was not calculated. Data for feed consumption and body weight were subject to statistical analysis using the students 't' test at the level of $p<0.05$.

Results and Discussion

The test is invalid if there is one non-incidental death or more than one death from any other cause. This did not occur as mortality in all groups was 0% therefore the experiment is considered valid.

Biological results

There was no incidental or non-incidental mortality recorded throughout the test period , there was also no signs of abnormal behaviour from any of the birds (Table B.9.1.1.1-1).

The pathology results showed that there were no abnormalities detected in the treatment group or either of the controls in any of the birds.

Table B.9.1.1.1-1 Mortalities and behaviour recorded throughout the test period

Dose [mg napropamide-M technical/kg body weight.	Dose volume [mL/kg body weight.	Number of birds/group (Female).	Mortality (up to 14 days) and visual symptoms in brackets.	% Mortality.
0 (Control)	10	5	0 (N)	0
0 (Vehicle control)	10	5	0 (N)	0
2000	10	5	0 (N)	0

Key: N = Normal behaviour.

Table B.9.1.1.1-2 shows that the body weights of the birds within each group were comparable before exposure occurred. No statistical difference was observed in mean body weight or mean body weight gain from the treated birds compared to the control and vehicle control groups.

Table B.9.1.1.1-2 Group mean body weight (g) along with standard deviation

Treatment group (mg napropamide-M /kg b.wt.)	Mean body weight (g)				Mean body weight gain (g)			Mean percent body weight change		
	0	3	7	14	0-3	4-7	8-14	0-3	4-7	8-14
0 (control)	248.2 ±29.1	296.4 ±34.2	272.2 ±33.9	273.0 ±35.0	21.2 ±19.6	2.8 ±9.3	0.8 ±6.5	8.64 ±8.46	1.14 ±3.93	0.28 ±2.42
0 (Vehicle control)	246.8 ±34.2	260.4 ±19.8	268.4 ±17.4	268.6 ±17.3	13.6 ±9.6	8.0 ±2.6	0.2 ±4.1	5.70 ±4.41	3.14 ±1.8	0.08 ±1.51
2000	244.2 ±20.3	247.2 ±36.4	254.2 ±30.1	268.8 ±32.6	3.0 ±24.7	7.0 ±11.8	14.6 ±14.7	1.06 ±10.25	3.25 ±5.28	5.84 ±6.25

Key: b.wt = Body weight

No statistically significant differences were observed ($p<0.05$) in feed consumption in the test item treatment group compared to the control and vehicle control groups (Table B.9.1.1.1-4).

Table B.9.1.1.1-4 Mean Feed Consumption (g/bird) throughout the test along with standard deviation

Dose [mg napropamide-M technical/kg body weight.	Feed Consumption (g/bird) on Days -		
	0-3	4-7	8-14
0 (Control)	205.6 ±43.2	264.4 ±151.0	393.2 ±177.8
0 (Vehicle control)	153.0 ±21.9	235.0 ±96.0	567.0 ±371.3
2000	141.4 ±66.9	248.6 ±163.3	471.0 ±366.9

Key: b.wt = body weight

Conclusions

The acute oral LD_{50} of napropamide-M technical to Japanese quail was greater than 2000 mg/kg body weight.

RMS Comments

The study was conducted in line with OECD 223 under GLP compliance; all validity criteria were met. The study is considered acceptable for use in the risk assessment, with an LD_{50} of >2000 mg a.s./kg bw.

Several slight deviations were noted and these are discussed further below:

It is noted that OECD guideline (223) specifies that a cage space of 1000 cm^2 should be provided for each quail however only 900 cm^2 has been provided. The study report does not state the acclimatisation mortality, breeding history of the birds or phenotypes chosen for the test. The report states that the birds were observed continuously for the first two hours of the experiment however it is not specified if any incidents of regurgitation occurred. The guideline specifies that 'regurgitation compromises the evaluation of toxicity and should be recorded' as this is an important consideration of if the test animals were exposed to the full dose or the test item, this will be considered further in the risk assessment. Given that the validity criteria have been met, it is accepted that these devaitions would not impact the outcome of the study.

B.9.1.1.2. Short-term dietary toxicity to birds

With reference to EFSA guidance (EFSA Journal 2009; 7(12):1438) this test is no longer required. According to Regulation 283/2013, a study shall only be required where the mode of action or results from mammalian studies indicate a potential for the dietary LD_{50} measured by the short-term dietary toxicity study to be lower than the LD_{50} based on an acute oral study. No data is submitted. XDE-777 exhibits very low acute oral and dietary toxicity in mammals. There is no indication in the results of the mammalian studies that the potential dietary LD_{50} would be lower than that based on the acute oral LD_{50}. Therefore, a short-term dietary toxicity study in birds is not required and no data is submitted.

B.9.1.1.3 Sub-chronic toxicity and reproduction to birds

The following studies performed with racemic napropamide have been submitted to support the inclusion of napropamide-M. They were submitted and evaluated during the EU review of napropamide. In the interests of animal welfare, the RMS has accepted these studies for use in the risk assessment with a NOEL of 309 mg a.s./kg bw day ([redacted] 1991a) and 392 mg a.s./kg bw day (Beavers *et al*. 1991b). The studies have not been re-evaluated. The previously agreed Annex I endpoints have been used, where relevant, in the risk assessment.

Report: [redacted] (1991a) napropamide: a one-generation reproduction study with the bobwhite (*Colinus virginianus*). [redacted] Unpublished report No.: 123-159

Guidelines: US EPA FIFRA Guideline 71-4 (*Guideline deviations:* None stated)

GLP: Yes (40 CFR Part 160)

Material and methods: The objective of the study was to evaluate the effects of napropamide administered via the diet to bobwhite quail (*Colinus virginianus*) over a 22 week exposure period.

Test substance: Technical Devrinol, 95.2% measured purity, lot no. # 12031-2301

Test species: Bobwhite quail (*Colinus virginianus*) 25 weeks old, approaching their first breeding season and with average body weight of males at 198 g and females 192 g.
Test concentrations in diet: Nominal 0, 300, 1000 or 3000 mg as/kg feed. Mean measured diet concentrations ranged between 97% and 101% of nominal values.

Test design: Four treatment groups, each comprising 16 pairs of birds (one pair per pen) were fed diets *ad libitum* containing napropamide at the four treatment levels for 22 weeks.
Observations and sampling: All birds were observed daily for mortality, abnormal behaviour and signs of toxicity and body weight and feed consumption was measured throughout the experiment. Eggs were collected daily from the onset of egg production and were set weekly for incubation; the first eggs were set during Week 13. Throughout the laying period, eggshell thickness measurements were recorded. In addition, effects upon egg production and quality, hatchling health and survivability were examined.

Statistical analysis: Dunnetts multiple comparison procedure for comparing several treatments with a control was used to determine statistically significant differences between the control group and each of the treatment groups.

Test conditions: After 14 weeks of acclimation, exposure was initiated by a 7-week period of pre-photostimulation followed by a 3 weeks of pre-egg laying and 11 weeks of egg laying. For the first 7 weeks of the test, the birds were held under a photoperiod of 8 hours of light per day. During Week 8, the photoperiod was increased to 17 hours of light per day to induce egg laying; the photoperiod remained at 17 hours light per day until adult sacrifice. Temperature was 21.1 ± 3.0 °C and relative humidity 45 ± 14% (average and standard deviation). Pens were constructed of galvanized wire grid and galvanized sheeting. napropamide was mixed into the diet. Eggs were collected daily and stored until the weekly incubation at 13.4 ± 1.2 °C and 45 ± 7% relative humidity. All eggs to be incubated were fumigated with formaldehyde gas prior to incubation at 37.5 ± 0.1 °C.

All birds that died were necropsied and the pen mate was sacrificed and necropsied as well. The reproductive data from these pens were not included in the statistical analysis.

Food: Feed and water were provided *ad libitum* throughout the acclimation and testing period.

Results:

Table B.9.1.1.3-1: Reproduction toxicity of napropamide to bobwhite quail: Egg production, numbers of cracked eggs and survival results

Parameter	**Treatment (mg as/kg diet)**			
	Control (0)	**300**	**1000**	**3000**
Eggs laid	635	602	514	707
Eggs laid/female	40	46	43	47
% Cracked eggs	3	2	1	3
Number of viable embryos	490	502	425	562
Number of hatchlings	424	456	407	537
Number of 14-day old survivors	406	429	356	473
14-day old survivors/female	25	33	30	32

Table B.9.1.1.3-2: Reproduction toxicity of napropamide to bobwhite quail: Egg shell thickness data and mean chick body weights

Parameter	**Treatment (mg as/kg diet)**			
	Control	**300**	**1000**	**3000**
Mean egg shell thickness (mm)	0.213	0.212	0.206	0.208
Initial mean duckling bodyweights (g)	6.3	5.9	6.0	6.1
14-day survivors, mean body weights (g)	25	23	23	24

The above differences are not statistically significant from the control

Table B 9.1.1.3-3

Test compound	**napropamide (mg as/kg feed)**	**napropamide mg as/kg body weight/day[1]**
Test animal	Bobwhite quail	Bobwhite quail
LOEC	-	-
NOEC/D (adult symptoms)	3000	309
NOEC/D (reproduction)	3000	309

-: No information in report.
1: Calculations based on the conversion calculation detailed in guidance document SANCO/4145/2000, draft version 25 September 2002. Average body weight weeks 0-22: ♀= 213 g/bird, ♂ = 201 g/bird; Feed consumption week 0-22 = 21 g feed/bird/day at 3000 mg a.s./kg feed.

Exposure of bobwhite quail to napropamide treatment for 22 weeks at nominal concentrations of 0, 300, 1000 and 3000 mg as/kg diet did not result in any treatment-related mortalities, overt signs of toxicity or treatment related effects on adult body weight or feed consumption. There were no treatment-related effects upon reproductive parameters at any of the concentrations tested. Therefore, no LOEC or LC_{50} could be established and the NOEC-value is equal to the highest concentration tested.

Reviewer's assessment: The study was not compared to the specified guideline but it is well documented. In general it complies with the requirements of OECD Test Guideline 206 Avian Reproduction Test. Minor deviations (age at start of 25 weeks instead of 20-24 weeks and a prestimulation period of 7 weeks instead of 8 weeks) are not considered to be important. Only three concentrations were tested instead of the recommended five but, because there were no effects at any concentration, this does not influence the result. The calculation of the daily intake rate is in accordance with the guidance document. Therefore, the NOEC = 3000 mg/kg feed and NOED =309 mg as/kg body weight/day are considered to be valid.

Report: [redacted] (1991b) napropamide: a one-generation reproduction study with the mallard (*Anas platyrhynchos*). [redacted] Unpublished report No.: 123-160

Guidelines: US EPA FIFRA Guideline 71-4 (*Guideline deviations:* None stated)

GLP: Yes (40 CFR Part 160)

Material and methods: The objective of the study was to evaluate the effects of napropamide administered via the diet to mallard duck (*Anas platyrhynchos*) over a 19 week exposure period.

Test substance: Technical Devrinol, 95.2% measured purity, lot no. # 12031-2301

Test species: Mallard duck (*Anas platyrhynchos*) 36 weeks old, approaching their first breeding season and with average body weight of males 1247 g, females 1097 g.

Test concentrations in diet: Nominal 0, 300, 1000 or 3000 mg as/kg feed. Mean measured diet concentrations ranged between 97% and 101% of nominal values.

Test design: Four treatment groups, each comprising 16 pairs of birds (one pair per pen) were fed diets *ad libitum* containing napropamide at the four treatment levels for 19 weeks.
Observations and sampling: All birds were observed daily for mortality, abnormal behaviour and signs of toxicity and adult body weights were measured at test initiation on weeks 2, 4, 6 and 8 and at terminal sacrifice. Feed consumption was measured throughout the experiment. Eggs were collected daily from the onset of egg production and were set weekly for incubation. Throughout the laying period, eggshell thickness measurements were recorded. In addition, effects upon egg production and quality, hatchling health and survivability were examined.

Statistical analysis: Dunnetts multiple comparison procedure for comparing several treatments with a control was used to determine statistically significant differences between the control group and each of the treatment groups.

Test conditions: After 20 weeks of acclimation, exposure was initiated by a 9-week period of pre-photostimulation followed by 10 weeks of egg laying. For the first 9 weeks of the test, the birds were held under a photoperiod of 6 hours of light per day. The photoperiod was increased to 17 hours of light per day during week 10 to induce egg laying. The adults were maintained at a photoperiod of 17 hours of light per day until sacrifice. Temperature was 21.0 ± 2.4°C and relative humidity 57± 15% (average and standard deviation). Pens were constructed of galvanized wire grid and galvanized sheeting. Napropamide was mixed into the diet. Eggs were collected daily and stored until the weekly incubation at 13.4 ± 1.3 °C and 46 ± 8% relative humidity. All eggs to be incubated were washed with a chlorine based detergent prior to incubation at 37.5 ± 0.1 °C.

All birds that died were necropsied and the pen mate was sacrificed and necropsied as well. The reproductive data from these pens were not included in the statistical analysis.

Food: Feed and water were provided *ad libitum* throughout the acclimation and testing period.

Results:

Table B.9.1.1.3-4: Reproduction toxicity of napropamide to mallard duck: Egg production, numbers of cracked eggs and survival results

Parameter	Treatment (mg as/kg diet)			
	Control	**300**	**1000**	**3000**
Eggs laid	744	779	731	712
Eggs laid/female (mean)	50	52	49	47
% Cracked eggs	2	3	1	1
Number of viable embryos	580	584	561	547
Number of hatchlings	449	442	457	406
Number of 14-day old survivors	444	433	455	404
14-day old survivors/female (mean)	30	29	30	27

The above differences are not statistically significant from the control

Table B.9.1.1.3-5: Reproduction toxicity of napropamide to mallard duck: Egg shell thickness data and mean chick body weights

Parameter	Treatment (mg as/kg diet)			
	Control	**300**	**1000**	**3000**
Mean egg shell thickness (mm)	0.378	0.386	0.390	0.379
Initial mean duckling bodyweights (g)	37	40	40	41
14-day survivors, mean body weights (g)	280	277	271	271

The above differences are not statistically significant from the control

Table B.9.1.1.3-6: Results of one generation reproductive study with mallard duck

Test compound	**napropamide (mg as/kg feed)**	**napropamide mg as/kg body weight/day[1]**
Test animal	Mallard duck	Mallard duck
LOEC	-	-
NOEC/D (adult symptoms)	3000	392
NOEC/D (reproduction)	3000	392

-: No information in report.
1: Calculations based on the conversion calculation detailed in guidance document SANCO/4145/2000, draft version 25 September 2002. Average body weight weeks 0-22: ♀= 1150 g/bird, ♂ = 1208 g/bird; Feed consumption week 0-22 = 154 g feed/bird/day at 3000 mg a.s./kg feed.

Exposure of mallard duck to napropamide treatment for 19 weeks at nominal concentrations of 0, 300, 1000 and 3000 mg as/kg diet did not result in any treatment-related mortalities, overt signs of toxicity or treatment related effects on adult body weight or feed consumption. There were no treatment-related effects upon reproductive parameters at any of the concentrations tested. Therefore, no LOEC or LC_{50} could be established and the NOEC-value is equal to the highest concentration tested.

Reviewer's assessment: The study was not compared to the specified guideline but it is well documented. In general it complies with the requirements of OECD Test Guideline 206 Avian Reproduction Test. Only three concentrations were tested instead of the recommended five but, because there were no effects at any concentration, this does not influence the result. The calculation of the daily intake rate is in accordance with the guidance document. Therefore, the NOEC = 3000 mg/kg feed and NOED =392 mg as/kg body weight/day are considered to be valid.

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

A study to address the data requirement for acute oral toxicity to mammals has been conducted with napropamide-M, please refer to the DAR of napropamide-M, Volume 3 – B6 (AS), section B.6.2.1.

Table B.9.1.2.1-1:Summary of acute toxicity end point for mammals

Species	Test substance	Study type	Toxicity (mg/kg bw/day)	Toxicity (mg/kg feed)	References
Rat	a.s.	Acute	> **2000** Mortality	NA	[redacted] (2010)

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

Bridged data from a study conducted with napropamide was used to assess the reproductive toxicity of napropamide-M to mammals. These data have been assessed and accepted by the RMS mammalian toxicology specialist, and a NOEL of 30 mg a.s./kg bw day, has been confirmed based on the rat three generation study. Therefore this study has not been re-evaluated by the RMS and the previously agreed Annex I endpoints have been used.

Table B.9.1.2.2-1:Summary of reproductive toxicity end points for mammals

Species	Test substance	Study type	Toxicity (mg/kg bw/day)	Toxicity (mg/kg feed)	Reference
Rat	a.s.	Three generation study	**30**	Not reported	[redacted] (1978)

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

See section B.9.2.1.1 in the CP dossier.

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

No data submitted.

B.9.1.5. Potential for endocrine disruption

Currently, discussions are ongoing within the European Commission on how to address the issue of endocrine disruption. Until these discussions have concluded and a clear approach has been agreed with the Commission and the Member States, the RMS proposes no further consideration.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

The acute toxicity of D-napropamide, equivalent substance to napropamide-M, to rainbow trout has been assessed and a study summary provided below.

Report

[redacted] (2011a) D-napropamide: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour test. [redacted], Unpublished report No.: D03458

Guidelines

Directive 92/69/EEC, C.1; OECD 203 (1992), USA EPA OPPTS 850.1075

GLP

Yes (certified laboratory)

Materials and Methods

Test material	D-napropamide
Description:	Light brown crystalline powder
Lot/Batch #:	UPV/714-181/DEV/014
Purity:	97.2% w/w (total D- + L-isomer)
Stability of test compound:	Stable under test conditions (test performed as far as possible in the dark)
Solubility in water:	0.039 g/L at 20°C
Reference substance:	Not stated
Test animals	
Species:	*Oncorhynchus mykiss*
Description:	Mean body length 5.1 ± 0.19 cm, mean body wet weight was 1.0 ± 0.06 g
Source:	[redacted]
Test dates	31st of January 2011 – 16th of February 2011

Test Design

Five groups of seven fish were exposed to D-napropamide for 96 hours in a static system without test medium renewal. The fish used in this study was rainbow trout obtained from a fish breeding station in Germany. The mean body length of the fish was 5.1 ± 0.19 cm and the mean body wet weight was 1.0 ± 0.06 g (Mean ± SD) (values based on measurement of 10 fish at test start).

For the acclimation period the fish were held for more than twelve days without any medication. Prior to the start of the test the fish were held for one week in the test water which was maintained at the test temperature. During holding until two days before the start of the test, the fish were fed with a commercial fish diet. During the last three weeks prior to the test, no fish died in the test fish batch and all fish were healthy.

Due to the low solubility of the test item in reconstituted test water, a dispersion of the test item with the loading rate of 100 mg/L was continuously stirred at room temperature in the dark for over 3 hours. Then, the dispersion was filtered. The resulting test medium (undiluted filtrate) was used as the highest concentrated test medium and as a stock solution for preparation of the test media with lower test concentrations. For this preparation the filtrate was serially diluted with test water to give dilutions of 1:2.2, 1:4.6, 1:10 and 1:22. The test media were prepared just before introduction of the fish i.e. at start of exposure. The selection of concentrations was based on the results of a non GLP range finding test. Additionally, a test water control without test item was also tested. The duplicate samples of the test medium from the three highest test concentrations from all sampling times (0, 48 and 96 hours) or

from the last sampling time when all fish were dead were analysed via HPLC with UV-vis detection method.

One glass test vessel wrapped with black plastic foil with 15 litres of test medium was used for each treatment. Reconstituted test water was used in the study, the water temperature (measured at test start and once daily) was 13 – 14 °C. Dissolved oxygen (measured in each vessel at the start and once daily) was 9.2 to 9.9 mg/L. The pH of the test media (measured in each vessel at the start and once daily) was 7.2 to 7.4. The hardness of the water was 125 mg $CaCO_3$ per litre and the ratio of Ca:Mg and Na:k was 4:1 and 10:1 respectively. The test water was aerated prior to the preparation of the test media until oxygen saturation was reached. The appearance of the test media was also recorded at the same time. As the test item is not stable under light conditions the test was carried out as far as possible in the dark to avoid a photolytic degradation of the test item.

The fish were introduced into each test vessel in a random order and the loading rate was 0.48 g fish wet weight per litre of test medium. The fish were not fed during the test. The test fish were observed for mortality and visible abnormalities after approximately 3, 24, 48, 72 and 96 hours test duration. The study report does not state how mortality was determined.

For the analysis of the results the LC_{50} could not be calculated by Probit Analysis or Moving Average Interpolation due to the steep concentration-effect relationship. Instead, the LC_{50} values were determined as a geometric mean value of the two consecutive test concentrations with 0 and 100% mortality and the 95% confidence limits were the concentrations with 0 and 100% mortality. The NOEC was determined directly from the raw data.

Results and Discussion

All of the validity criteria were met. The mortality in the control was 0%. Constant conditions were maintained throughout the test and the concentration of the test substance was 91 to 99% of nominal. The dissolved oxygen concentration was 9.2 mg O2/L.

Analytical results

At the start of the test, the analytically determined concentrations of D-napropamide in the test media were 7.9, 16.8 and 35.7 mg/L, respectively. At the end of the respective exposure period, the mean measured concentrations of D-napropamide (calculated as the arithmetic mean of the stated concentrations) in the test media were 7.5, 17.0 and 36.0 mg/L, respectively, indicating that concentrations of the test substance were within 91 to 99% of the initially measured concentrations. Thus, the test item was stable over the test period of 96 hours.

Biological Results

All reported biological results are based on mean measured test item concentrations, calculated as the arithmetic means of the concentrations measured at the start of the test.

In the control and at the test concentrations up to and including a mean measured concentration of 7.5 mg a.s./L, all fish survived until the end of the test and no visible abnormalities were observed in the test fish. At the two highest concentrations of 17 and 36 mg/L, all test fish were dead after 24 and 3 hours of test duration, respectively.

Table B.9.2.1-1 Mortality and visible abnormalities observed in the test fish after exposure to D-napropamide for 96 h.

Treatment group initial measured (mg a.s./L)	**Mean measured concentration (mg a.s./L)**	**Number of abnormal and dead fish / number of dead fish** *Type of visible abnormalities*				
		Observation time				
		3 h	**24 h**	**48 h**	**72 h**	**96 h**

Treatment group initial measured (mg a.s./L)	Mean measured concentration (mg a.s./L)	Number of abnormal and dead fish / number of dead fish *Type of visible abnormalities*				
		Observation time				
		3 h	24 h	48 h	72 h	96 h
Control (test medium only)	-	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
na	n.a.	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
na	n.a.	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
7.9	7.5	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
16.8	17.0[#]	7 / 0 *SR*	7 / 7	-/-	-/-	-/-
35.7	36.0[##]	7/7	-/-	-/-	-/-	-/-

n.a.- not analysed
#: based on the measurement of 24 hours as 100% mortality had occurred 24 hours following exposure
##: based on the measurement of 0 hours only as 100% mortality recorded at three hours following exposure.
-/-: all fish dead
SR: Fish lying on side or back on the bottom
(the LC_{50} values were determined as a geometric mean value of the two consecutive test concentrations with 0 and 100% mortality, and the 95% confidence limits for the LC_{50} as the test concentrations with 0 and 100% mortality. The NOEC, LOEC, LC0 and LC100 were determined directly from the raw data).

Conclusion

Based on mean measured concentrations, the 96-hour NOEC of D-napropamide to rainbow trout was determined to be 7.5 mg a.s./L. The 96-hour LC_{50} of D-napropamide was calculated to be 11.2 mg a.s./L with a 95% confidence interval of 7.5-17 mg/L.

RMS Comments

The study was conducted in line with OECD 203 under GLP compliance: all validity criteria were met. The RMS considers that the study is suitable for use in the rsik assessment. The LC_{50} was 11.2 mg a.s./L and the NOEC is 7.5 mg a.s./L.

OECD guideline 203 specifies that 'fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction', however the study report doesn not state the method used to determine mortality.

It is also noted that the definitive study was conducted under complete darkness due to concerns with the stability of test item'. This is a deviation to the guideline which specifies that '12 to 16 hours photoperiod daily' should be implemented. Complete darkness has been demonstrated to be unneccesary in other aquatic studies with the test item and may influence the movement of the fish in water and therefore exposure. However as no abnormal behaviour was observed in the control and all validity criteria were met it is accepted that this deviations would not have an adverse effect on the outcome of the study.

The mean measured concentrations should have been calculated in terms of the geometric mean as the possible ranges of active substance recovery at the sampling periods are not independent of each other. The RMS therefore requested a re-calculation by the applicant. The results are as follows :

Table B.9.2.1-2: Comparison of arithmetic and geomean measured concentrations in the fish acute study

Age (day)	Loading rate of 100 mg/L	Mean measured concentration of the test item (arithmetic mean)	Mean measured concentration of the test item (geometric mean)

0	Control	< LOQ	< LOQ
	Dilution 1:4:6	7.90	7.90
	Dilution 1:2.2	16.8	16.78
	Filtrate undiluted	35.7	35.70
1	Dilution 1:2.2	16.6	16.60
2	Dilution 1:4.6	7.47	7.47
4	Control	< LOQ	< LOQ
	Dilution 1:4.6	7.18	7.18

[a] based on loading rate of 100 mg/L
 # based on the measurement of 24 hours as all fish were dead
based on the measurement of 0 hours only as all fish were dead after 3 hours

The calculated geomean values of the measured concentrations are only marginally different to the arithmetic mean measured concentrations and as such the UK agrees that the calculation of the effect concentrations are not affected.

The lowest two dilutions (1:22 and 1:10) were not subject to analytical verification of the test substance. Therefore, it is not known if these nominal concentrations were achieved or maintained within 80-120% throughout the test. This could potentially mean that the test organisms were exposed to below the stated concentrations. As a result, there is uncertainty in the endpoint proposed by the applicant as the stability of the test item is not known. If all concentrations were not maintained within 80-120% of the nominal at all sampling points then an LC_{50} based on mean measured concentrations could potentially be lower. However, as the determined LC_{50} of 11.2 mg a.s/L fell within the three highest concentrations that were subject to analytical verification the evaluator considers that this endpoint is suitable for use in the risk assessment.

B.9.2.2. Long-term and chronic toxicity to fish

In accordance with Regulation (EU) No. 283/2013 an assessment of the chronic toxicity to fish is required by either an early life stage toxicity assessment or full life cycle assessment. An early life stage study has been conducted with napropamide-M and a summary provided below.

Report

[redacted] (2015a) Assessment of the effect of napropamide-M on fish, early life stage toxicity test. [redacted], Unpublished report No.: ENV-13-040

Guidelines

OECD guideline 210, Fish early life stage toxicity test (2015).

GLP

Yes (certified laboratory)

Materials and Methods

Test material	Napropamide-M technical
Description:	Orange-white crystals
Lot/Batch #:	UPH-08/DNE-263/TECH/20121226.
Purity:	97.26 % w/w (total D- + L-isomer)
Stability of test compound:	Stable under test conditions
Solubility in water:	0.039 g/L at 20^{o}C
Reference substance:	N/A

Test animals	
Species:	Zebrafish *(Danio rerio).*
Age:	Embryonic – (3 – 4 hours post fertilisation)

Source: [redacted].

Experimental dates: 24th of April 2014 – 17th of June 2014.

Test Design

The test was conducted using *Danio rerio* taken from an on-site culture. The broodstock were maintained in cultures at the facility which were kept under conditions similar to those used for the definitive test period. In the main study embryonic zebrafish at the '1k-cell' to 'sphere' stage were exposed to five concentrations of the test item (0.05, 0.14, 0.40, 1.09 and 3.00 mg a.s. L^{-1}) as well as control and solvent control groups. All test groups consisted of 4 replicate tanks with 20 embryos per replicate.

Test vessels consisted of glass tanks containing a nominal volume of 10L. The water was aerated and heated in a sump to bring it within the condition parameters required by the guideline for zebrafish. After the pH, dissolved oxygen and temperature had stabilised the water was pumped into a glass header. The embryos were carefully transferred by pasteur pipette onto glass watch glasses which were placed under the tank inflow which provided a flow of test item solution over the embryos. When the larvae hatched they were able to swim off the watch glasses and locate themselves around the tank.

Embryos were exposed to napropamide-M from approximately 3-4 hours post-fertilisation until 30 d post-hatching. The hatching success of the embryos was determined and subsequent survival to 30 days post hatching was assessed. Hatching success was recorded daily until all embryos were recorded as hatched or dead. Any embryos, larvae or juvenile fish that were observed showing abnormal behaviour (e.g. hyperventilation, uncoordinated swimming, atypical quiescence or change in feeding behaviour) or appearance (e.g. body form) were recorded daily, any larvae that were static at the bottom of the tank were stimulated by gently moving them with a plastic Pasteur pipette. If larvae did not respond to this they were removed, checked for signs of life and disposed of when mortality was confirmed. At the end of the test individual weight and length data were recorded. This was achieved by blotting dry all individuals from one tank on filter paper after they had been euthanised. The fish were then weighed individually to get a fish wet weight. The fish were also individually measured for total length.

Temperature was monitored continuously in one of the control tanks for the duration of the test period, the temperature in all test tanks was measured every other day (23.6 to 27.3 ºC). The photoperiod was 16 hours. The pH (7.77– 8.16) and dissolved oxygen (45.4 – 100% ASV) in all test tanks were measured every other day. For the last few days of the test it was necessary to provide some aeration in the fish tanks as the fish were growing larger and becoming more active. In the diluent water and one control tank the total ammonia (0.05 – 0.12 and 0.03 – 0.08 mg L^{-1}), nitrite (0.00 – 0.02 and 0.00 – 0.02 mg L^{-1}) and nitrate (55.72 - 71.35and 52.03 – 70.88 mg L^{-1}) were measured every 7 days. Total hardness was measured at the beginning and end of the experiment in the diluent and the control (both were 220 -225 mg CaCO3 L^{-1}). All fish tanks were cleaned every day to remove any detritus or excess feed in the water or on the tank surfaces.

Food was provided to all tanks from day 0 onwards, for days 0 to10 the test animals were given <100 micron dry food three times a day, for days 11 to 18 they were given <100 micron dry food three times a day and 24 h brine shrimp three times and day and for days 19 to 30 they were given <100 micron dry food three times a day and 48 h brine shrimp three times and day.

The concentrations of all the test item solutions were determined at regular intervals throughout the test period (days-2, -1, 0, 1, 2, and then every Monday and Thursday throughout the test period). The samples were analysed using an external standard high performance liquid chromatography (HPLC) method. The flow rates of the mixer vessel overflows for a selection of tanks were checked at regular intervals to provide evidence for the flow through volume replacement rate. Rates were checked

approximately twice a week. The recorded flow rate was 50.4 L solution per 24 h which was equivalent to 5.4 volume replacements of the tank in a 24 h period.

For hatching success and post hatch survival treatment groups were compared to the pooled controls using Fisher's Exact test with Bonferroni-Holm correction ($\alpha = 0.05$), for weight and length treatment groups were compared to the pooled controls using Dunnett's multiple comparison test ($\alpha = 0.05$). As there were no statistically significant differences it was not possible to generate a regression analysis.

Results and Discussion

Analytical results

The concentration of all the test item solutions was determined at regular intervals throughout the test period. The samples were analysed using an external standard high performance liquid chromatography (HPLC) method. The recovery of the test item over the entire period was 82 -124 % of the nominal concentration added.

Validity criteria

The dissolved oxygen concentration was 45.4 – 100.5 % of the air saturation value throughout the test. The water temperature did differ by more than + 1.5^{o}C between test chambers or between successive days at any time during the test (23.6 to 27.3 ^{o}C) and was not within the temperature ranges specified for the test species (26 ± 1.5 °C).

The overall survival of fertilised eggs and post-hatch success in the controls and in the solvent controls was 90% in the control and 95% in the solvent control and 61.31% in the control and 67.27% in the solvent control respectively.

Biological Results

The results for hatching success, survival, weight and length are all represented as group means (*n* = 4). The post-hatch survival for all control and treatment groups throughout the test period was assessed and is summarised in table B.9.2.2-1.

Hatching success

The hatching success for all control and treatment groups was over 70%, hatching started approximately 48 hours post-fertilisation and was complete approximately 120 hours post-fertilisation. No treatment related effects were observed at concentrations ranging from 0.05 to 1.09 mg a.s./L. However, a statistically non-significant effect was noted at 3.0 mg a.s./L.

Table B.9.2.2-1 Mean hatch success and group mean survival for each treatment group (observations recorded daily) and group mean blotted wet weight and group mean total length (observations recorded at the end of the test period)

Treatment (mg a.s. L^{-1})	Mean hatch success (%)	Group mean survival (% of hatched)	Group mean blotted wet weight (mg)	Group mean total length (mm)
Control	90 (± 2.3)	61.91(± 15.07)	48.41(± 23.46)	18.7(± 3.35)
Solvent Control	95 (± 0.71)	67.27(± 6.11)	51.28(± 18.27)	19.06(± 2.53)
0.05	94(± 0.83)	75.44(± 18.45)	49.02(± 18.59)	18.62(± 3.01)
0.14	94(± 0.83)	54.33(± 22.88)	51.78(± 22.83)	18.81(± 2.95)
0.40	94(± 1.64)	50.97(± 27.12)	49.32(± 17.79)	18.77(± 2.34)
1.09	96(± 0.83)	71.54(± 10.37)	46.85(± 18.34)	18.11(± 3.10)
3.00	88(± 2.28)	50.56(± 14.55)	46.55(± 12.31)	18.06(± 1.49)

(*= significant result, Fisher's Exact test with Bonferroni-Holm correction, $\alpha = 0.05$)

Survivorship

In the controls, mortality of 33 to 40% was evident over the course of the study. Mortality occurred during the latter stages of the study, day 14 onwards. A statistically non-significant decrease in

survivorship was observed at concentrations of 0.14, 0.4 and 3.0 mg a.s./L, although this pattern was not evident at 1.09 mg a.s./L. The majority of mortality in the 3.0 mg a.s./L treatment group occurred between days 10 and 14 post-treatment Fry mortality in the 0.14 and 0.4 mg a.s./L treatment groups occurred in a single replicate. Overall though, there were no statistically significant differences in the number of fish surviving in any of the treatment groups compared with the controls.

Growth

For growth, weight and body length were determined at study termination. A statistically non-significant decrease in wet weight and total length was noted at concentrations of 1.09 mg a.s./L and above. In addition, various physical defects were noted at study termination; these are summarised in table B.9.2.2-2.

Table B.9.2.2-2 End of study (day 30 post-hatch) observations (numbers of fish exhibiting abnormal morphology or behaviour, all other fish were apparently healthy)

Treatment (mg a.s. L-1)	Opercula defect	Tail fin deformity	Spinal deformation	Small fish[1]	Very small fish[2]
Control	5	1	1		1
Solvent Control	3	1	1		1
0.05	3			2	
0.14	2	2		3	
0.40	3		1	2	
1.09	1	1		2	2
3.00					

[1]Small fish denotes a fish that was clearly smaller than the tank average. [2]Very small fish denotes a fish that had not grown significantly post-hatching.

Conclusion

The results showed that there were no significant effects on hatch success, post-hatch survival, fish weight or fish length after approximately 34 days of exposure to napropamide-M technical. The NOEC was therefore determined to be 1.09 mg a.s/L.

RMS Comments

A high number of deviations to the guideline occurred, including to a number of the validity criteria. The temperature was slightly out of the stated range for the zebra fish and varied more than 1.5 ºC because of this (23.6 to 27.3 ºC). The dissolved oxygen content fell to below 60% ASV (45.4%) and the post hatch success in the controls was below 75% (61.31% in the negative control and 67.27% in the solvent control). It is noted that the study report states that the temperature and dissolved oxygen content deviations were rectified quickly and only occurred in one replicate each and the post hatch success appeared low because the hatching success was so high (90% in the control and 95% in the solvent control), however as the majority of the validity criteria were affected this is not considered acceptable by the RMS.

The following deviations to the guideline were also noted:

- On two occasions the flow rate fell 16% below the rate specified in the guideline of 5 ± 0.5 tank replacements in 24 hours. The study report states that this was rectified quickly and it does not appear to have had a lasting effect.
- The concentration of the test item had on a few occasions risen to above 120% of the nominal
- The raw data also indicated that on one sampling period (day 19) the concentration in one of the tanks was recorded as 483% of the nominal due to a blocked diluent line. The study report states that this value was not used for the overall mean as it was an anomalous result which occurred because the diluent line was blocked and this issue was quickly rectified.

In addition, several results from the study were also a cause for concern:

- The following abnormal symptoms were recorded in the control: five fish with opercula defect, one tail fin deformity, one spinal deformity and one very small fish. This is a concern as it indicates that the batch of fish were not initially healthy.
- Increases in mortality were noted at 0.14 and 0.4 mg a.s./L. In both treatment groups, the increase was driven by mortality in a single replicate. At study termination, only 3 and 1 fish were alive in the 0.14 and 0.4 mg a.s./L treatment groups. This was not considered by the RMS to be treatment related. A sample of the survivorship data for the end of the study is provided below.

Table B.9.2.2-3 Sample of survival data

Day	**25**	**26**	**27**	**28**	**29**	**30**
0.014 (mg a.s./L)						
1	3	3	3	3	3	3
2	13	13	13	13	13	13
3	11	11	11	11	11	11
4	14	14	14	14	14	14
Mean	10.25	10.25	10	10.25	10.25	10.25
0.40 (mg a.s./L)						
1	14	14	14	14	14	14
2	2	2	2	2	1	1
3	10	10	10	10	10	10
4	1	15	15	15	14	15
Mean	10.25	10.25	10.25	10.25	9.75	10

- The report states that one very small fish was observed in the control however the definition of ‘very small fish’ has not been clearly quantified only that a very small fish denotes a fish that had not grown significantly post-hatching, it does not state if this was in relation to weight or length or both.

Due to the large number of guideline deviations, including a number of the validity criteri, the RMS has determined that the study not reliable. However with reference to Article 60 of Directive 1107 http://crdintranet/201500029/EC%20Legislation/Regulation%201107_2009%20Parliament%20Council%20%20concerning%20PPP.pdf), in order to minimise vertebrate testing a repeat study is not considered to be necessary. This will be considered further in the risk assessment.

The RMS has determined that the NOEC of >3.0 mg a.s./L is not acceptable. At the top two concentrations of 3.0 and 1.09 mg a.s./L, length/weight are reduced and there is some indication of a dose-response relationship. although these results are not statistically significant they may be biologically signinficant, therefore a NOEC of 0.4 mg a.s/L is more appropriate. With these considerations the study is considered suitable for use in the risk assessment.

B.9.2.3. Potential for endocrine disruption

Currently, discussions are ongoing within the European Commission on how to address the issue of endocrine disruption. Until these discussions have concluded and a clear approach has been agreed with the Commission and the Member States, the RMS proposes no further consideration.

B.9.2.4. Acute toxicity to aquatic invertebrates

A study to assess the acute toxicity of D-napropamide (equivalent to napropamide-M) to *Daphnia magna* has been conducted and summary provided below.

Report

A. Liedtke (2011c) D-napropamide: acute toxicity to *Daphnia magna* in a 48-hour immobilization test. UPL Europe Ltd, Unpublished report No.: D03447

Guidelines

Directive 92/69/EEC, C.2; OECD 202 (2004)

GLP

Yes (certified laboratory)

Materials and Methods

Test material	D-napropamide.
Description:	Light brown crystalline powder.
Lot/Batch #:	UPV/714-181/DEV/014.
Purity:	97.2% w/w (total D- + L-isomer)
Stability of test compound:	Stable under test conditions (test performed as far as possible
Solubility in water:	in the dark).
Reference substance:	0.039 g/L at 20^{o}C
	Potassium Dichromate
Test animals	
Species:	*Daphnia magna*
Strain:	Straus
Source:	Laboratory stock, Harlan Laboratories Ltd. Zelgliweg 1 4452 Itingen / Switzerland.
Experimental dates:	28th of December 2010 – 3rd of February 2011.

Test Design

The study was performed with young daphnids of the species *Daphnia magna* Straus. The animals used in the test have been bred in reconstituted water under temperature and light conditions identical to those of the tests. The parental daphnids were maintained in test water for at least 48 hours prior to the start of the test. During breeding the daphnids were fed three times a week with an algal suspension of the green algae *Desmodesmus subspicatus.*

The selection of the test concentrations was based on the results of a range-finding test to determine the dosage and stability of the test item in the test water (non-GLP). Based on the results of this six groups of twenty *Daphnia magna* (6 – 24 hours old) were exposed to D-napropamide (dilutions 1:2.2, 1:4.6, 1:10, 1:22, 1:46 and the undiluted filtrate) for 48 hours in a static system. Due to the low solubility of the test item in test water, a dispersion of the test item with the loading rate of 100 mg/L was continuously stirred at room temperature in the dark over 3 hours which was then filtered. For each treatment, 20 daphnids were used divided into four replicates of five daphnids each. The volume of test solution provided for each daphnid was 25 mL.

At the start and end of the test, the pH values (7.7 to 7.9), dissolved oxygen concentrations (8.2 to 8.5 mg/L) and water temperature (20-21 °C) were determined at each treatment. The appearance of the test media was visually recorded at the start of the test and after 24 and 48 hours. The test was

performed as far as possible in the dark to avoid a photolytic degradation of the test item. The daphnids were not fed during the test. Reconstituted test water was used in the study, to meet both the EPA- and OECD-requirements the hardness was lowered by a factor of 1.7 of the normal hardness. The reconstituted test water consisted of analytical grade salts dissolved in purified water. The immobility of the daphnids was determined by visual inspection after 24 and 48 hours of exposure. Those daphnids not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilized.

The samples of the test medium (8.5, 17.5 and 37.6 mg/L) were analysed via HPLC with UV/VIS detection method at test start and after 48 hours. For evaluation of the quality of the daphnia clone and the experimental conditions, potassium dichromate was tested as a positive control twice a year.

The 24-hour EC_{50} could not be calculated by Probit Analysis or Moving Average Interpolation due to the steep concentration-effect relationship. Instead, the 24-hour EC_{50} was calculated by linear interpolation of the immobility between the two consecutive test concentrations with 0 and 65% immobility on a semi-logarithmic scale (the 95% confidence limits could not be determined). The NOEC was determined directly from the raw data.

Results and Discussion

All of the validity criteria were met. The mortality in the control was 0% and no visual indications of stress were observed over the test period. The dissolved oxygen concentration was 8.2 to 8.5 mg/L throughout the test which meets the criterion of > 3mg/L.

Analytical results

At the start of the test, the analytically determined concentrations of D-Napropamide in the test media were 8.50, 17.5 and 37.6 mg/L, respectively. At the end of the test, values of 8.45, 17.1 and 37.1 mg/L, were found. Thus, the test item was stable over the test period of 48 hours.

The mean measured concentrations (calculated as the arithmetic mean of the stated concentrations) in the dilutions 1:4.6, 1:2.2 and the undiluted filtrate were 8.5, 17 and 37 mg/L respectively. The biological results were related to the mean measured concentrations of the test item.

Biological Results

For evaluation of the quality of the *Daphnia* clone and the experimental conditions, potassium dichromate was tested as a positive control twice a year. The result of the latest positive control test in October 2010 (48-hour EC50: 0.56 mg/L, study C97643) showed that the sensitivity of the test organisms was within the internal historical range (48-hour EC50 from 2000 to 2010: 0.43-1.1 mg/L).

All reported biological results are based on mean measured test item concentrations and are summarised in the table below.

During the first 24 hours of the test, no immobilized test organisms were determined in the control and up to and including the mean measured test item concentration of 17 mg/L. At the highest mean measured test concentration of 37 mg/L, the immobilization was 65% after 24 hours. After 48 hours of exposure, no immobilized test organisms were determined in the control and up to and including the mean measured test item concentration of 8.5 mg/L. At the concentration of 17 mg/L, 15% of the daphnids were found to be immobile. At the highest mean measured test concentration of 37 mg/L, all test organisms were found to be immobile. The 48-hour EC_{50} was calculated to be 19 mg/L with 95% confidence limits of 15 and 24 mg/L.

Table B.9.2.4-1 Number and percentage of immobilised *Daphnia magna* exposed to D-napropamide

Treatment group initial concentrations	Mean measured concentration (mg a.s/L)	Immobilised *Daphnia* (number)		Immobility of *Daphnia* (%)	
		24 h	48 h	24 h	48 h

Treatment group initial concentrations	Mean measured concentration (mg a.s/L)	Immobilised *Daphnia* (number)		Immobility of *Daphnia* (%)	
		24 h	48 h	24 h	48 h
Control (test medium only)	-	0	0	0	0
1:46	n.a.	0	0	0	0
1:22	n.a.	0	0	0	0
1:10	n.a.	0	0	0	0
8.5	8.5	0	0	0	0
17.5	17.0	0	3	0	15
37.6	37.0	13 (7)	20	65	100

Value in parenthesis: number of test animals with an adverse effect (daphnids moving only very slowly)
n.a.- not analysed

Conclusion

Based on mean measured concentrations, the EC_{50} value for immobilisation was calculated to be 19 mg test item/L at 48 hours. The NOEC (no observed effect concentration) at 48 hours was determined to be 8.5 mg test item/L.

RMS Comments

The study was conducted in line with OECD 202 under GLP compliance: all validity criteria were met. The RMS considers that the study is suitable for use in the risk assessment. The EC_{50} was 19.0 mg a.s./L and the NOEC is 8.5 mg a.s./L.

It is noted that the study does not consider the lowest three dilutions (1.46, 1:22 and 1:10) in the analytical verification of the test substance. Therefore, it is not known if these nominal concentrations were achieved or maintained within 80-120% throughout the test. This could potentially mean that the test organisms were exposed to below the stated concentrations. As a result, this there is uncertainty in the endpoint proposed by the applicant as the stability of the test item is not known. If all concentrations were not maintained within 80-120% of the nominal at all sampling points then an EC_{50} based on mean measured concentrations could potentially be lower. However as the determined EC_{50} of 19 mg test item/L fell within the three highest concentrations which had been subject to analytical verification (dilutions 1:4.6, 1:2.2 and the undiluted filtrate, equivalent to 8.5, 17 and 37 mg a.s/L, respectively), the RMS considers that this would not affect the outcome of the study.

The mean measured concentrations should have been calculated in terms of the geometric mean as the possible ranges of active substance recovery at the sampling periods are not independent of each other. The RMS therefore requested a re-calculation by the applicant. The results are as follows:

Table B.9.2.4-1: Comparison of arithmetic and geomean measured concentrations in the acute Daphnia magna study

Age (day)	Loading rate of 100 mg/L	Mean measured concentration of the test item (arithmetic mean)	Mean measured concentration of the test item (geometric mean)
0	Control	< LOQ	< LOQ
	Dilution 1:4:6	8.50	8.50
	Dilution 1:2.2	17.5	17.53
	Filtrate undiluted	37.6	37.60

2	Control	< LOQ	< LOQ
	Dilution 1:4:6	8.45	8.44
	Dilution 1:2.2	17.1	17.11
	Filtrate undiluted	37.1	37.10

The calculated geomean values of the measured concentrations are only marginally different to the arithmetic mean measured concentrations and as such the UK agrees that the calculation of the effect concentrations are not affected.

It is also noted that the difinitive study was conducted under complete darkness due to concenrns with the stability of test item. This is a deviation to the guideline which specifies that '12 to 16 hours photoperiod daily' should be implemented, in addition complete darkness has been demonstrated to be unnecessary in other aquatic studies with the test item. As all validity criteria were met and no abnormal behaviour was observed in the control it is accepted that this deviations would not have an adverse effect on the outcome of the study.

B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates

A study on the reproductive toxicity of napropamide-M to *Daphnia magna* has been conducted, a summary is provided below.

Report

M.K. Kamle (2014a) Reproductive output of *Daphnia magna* exposed to different concentrations of napropamide-M technical over a period of 21 days. UPL Europe Ltd, Unpublished report No.: 509-3-07-6174

Guidelines

OECD 211

GLP

Yes (certified laboratory)

Materials and Methods

Test material	Napropamide-M Technical
Description:	Beige colour crystalline solid
Lot/Batch #:	UPH-08/DNE-263/Tech/20121226
Purity:	97.26% w/w (total D- + L-isomer)
Stability:	Stable under test conditions.
Solubility in water:	0.039 g/L at 20^{o}C
Test animals	
Species:	*Water flea (Daphnia magna)*
Strain:	Straus.
Age:	First instar (≤ 24 hours).
Source:	Laboratory culture originating from a strain supplied by Department of Zoology, University of Pune, India.
Experimental Dates:	10th of September 2013 – 17 of February 2014.

Study Design

The daphnids were cultured and maintained in reconstituted water medium. They were fed with unicellular alga, *Pseudokirchneriella subcapitata*. For the acclimation period, healthy adult daphnids were held under the same conditions as those used in the main test, first instar daphnids (24 h old) were separated as they were produced and used for the study.

The nominal test concentrations used in the difinitive test were 0.1, 0.3, 1.0, 3.3 and 10.5 mg/L along with a water treated control. The test organisms were exposed for 21 days in a semi-static test with the test medium being changed at regular intervals of 48 h, on days up to day 21 of the study. Ten daphnids were tested at each concentration, they were held individualy in a glass beaker (600 mL capacity) containing 125 mL test solution.

The observations of the number of offspring produced in each replicate were conducted daily during the exposure period of 21 days. The numbers of live and dead offspring produced during the experimental period were recorded and removed from the glass beakers. The adults were observed for mortality, if any, throughout the duration of the experiment on a daily basis. Daphnia length was recorded on day 21.

Temperature (18.9 ± 21.1 ºC) , dissolved oxygen (8.64 -8.77 mg/L) and pH (7.52- 7.66) of the test media were measured initially (0 h) and after every 48 h by using a dissolved oxygen meter and pH meter before every medium renewal. The experiment was conducted in a water bath with a water temperature of 19.8 °C. Uniform temperature in the water chamber of the water bath was obtained by maintaining a slow circulation of water by a motor. Total hardness of the test media was measured prior to every medium renewal (196 to 208 mg/L as CaCO3). A photoperiod of 16 h light and 8 h dark was maintained using an automatic timer and light was provided using fluorescent tubes. Light intensity was measured during the exposure period (1120 to 1270 lux). Reconstituted water was used as the test media.

Exposure concentrations were analysed by HPLC with UV detection and ratio of isomers was determined by HPLC with chiral column, the lowest concentration and highest concentration were drawn at days 0 (Fresh), 2nd (aged), 10th (Fresh), 12th (aged), 18th (Fresh) and 20th (aged) The samples were analysed on the same day without any storage.

For the interpretation of the results the EC_{50} value for immobilisation (mortality) of the parental organisms were calculated according to the Probit analysis method. (Finney, 1971). The live birth index and survival index for each group starting from day of appearance of first brood to the day of termination were calcultated according to the guideline. Length of *Daphnia* was recorded on Day 21 and was subjected to statistical analysis.

Results and Discussion

All validity criteria were met. In the control the mortality of the parent animals was 0%. The mean number of offspring produced by living parent at the end of the test was 77.5.

The coefficient of variation around the mean number of living offspring produced per parent animal in the control was 15.5 indicating that the results are reliable.

Analytical results

Exposure concentrations were analysed by HPLC with UV detection and ratio of isomers was determined by HPLC with chiral column the results show that the measured concentrations ranged from 95.14 – 99.18% of nominal concentrations therefore then endpoinds can be based on the intial concentrations.

Biological results

The cumulative number of offspring produced in the control and at the test concentrations of 0.1, 0.3, 1.0, 3.3 and 10.5 mg a.s./L were 775, 770, 678, 594, 518 and 349 respectively. No dead offspring were observed in any of the test concentrations throughout the exposure and there was no adult mortality observed in any of the test concentrations. The mean numbers of offspring survivors per adult in the control group was 77.50 at the end of the experiment. The live birth index was found to be 100% at all of the test concentrations and control.

Length of *Daphnia* was recorded on Day 21 and it was observed that there was a dose-dependent decrease in *Daphnia* length compared with the control group. That was statistically significant at test concentrations of 1.0, 3.3 and 10.5 mg napropamide-M /L.

The total numbers of offspring per group were recorded and analysed statistically. There was a significant decrease ($p \leq 0.01$) in the production of offspring during the exposure period at 1.0, 3.3 and10.5 mg a.s./L compared with the control.

Table B.9.2.5-1 Number of live young, offspring, adult mortality and Daphnia length.

Concentration (mg napropamide-M /L)	Total numbers of live young produced	Offspring			Live Birth Index (%)	Percent Adult Mortality (%)	Mean Daphnia length (mm) on day 21.
		Mean	SD	N			
Control	775	77.50	11.79	10	100.0	0	3.7
0.1	770	77.00	9.67	10	100.0	0	3.5
0.3	678	67.80	8.95	10	100.0	0	3.4
1.0	594	59.40↓↓	4.84	10	100.0	0	2.9, ↓↓
3.3	518	51.80↓↓	8.27	10	100.0	0	2.7, ↓↓
10.5	349	34.90↓↓	4.04	10	100.0	0	2.6, ↓↓

SD = Standard Deviations; N= Number of Adults, ↓↓ = significantly lower than control ($p \leq 0.01$)

Conclusions

The 21-day NOEC based on both survival and reproduction was 0.3 mg napropamide-M/L.

RMS Comments

It is noted that OECD guideline (211) specifies that observations of abnormal behaviour should be reported however the study report does not contain this information. It is also noted that a solvent wasn't used even though the substance is known to be poorly soluble in water. As no signs of precipitation were reported and the analytical verification was within the expected range, this is not considered to have affected the outcome of the study.

This study was conducted in line with OECD 211, under GLP compliance; all validity criteria were met. The RMS considers that this study is suitable for use in the risk assessment with a NOEC of 0.1 mg a.s./L.

B.9.2.6. Effects on algal growth

The following study performed with napropamide is provided in support of the assessment (ErC_{50} = 55.0 mg a.s./L). It was also submitted and evaluated during the EU review of napropamide. Therefore this study has not been re-evaluated by the RMS and the previously agreed Annex I endpoints have been used, where relevant, in the risk assessment.

REFERENCE: Annex IIA 8.2.6 / 02

Report: C.A. Jenkins (2002a) napropamide: algal growth inhibition assay (*Anabaena*). Huntington Life Sciences Ltd., Unpublished report No.: UPH021/013213.

Guidelines: OECD Guideline 201 (1984) and EC Methods, Part C, Method 3 (*Guideline deviations: None stated)*

GLP: Yes (certified laboratory).

Material and methods: The purpose of the study was to determine the inhibition of algal growth by napropamide.

Test substance: Technical napropamide, 93.2% w/w measured purity, batch No.: KJD 0602.

Test species: *Anabaena*, added to give a nominal cell concentration of 1 x 10^4 cells/mL.

Test concentrations: 0, 4.27, 9.39, 20.7, 45.5 and 100 mg/L. Measured concentrations of the test substance ranged between 28 and 98% at the start of the exposure period and were between 74 and 137% of the nominal exposure concentrations at the end of the exposure period. Therefore geometric mean measured values were used to calculate the results.

Test design: Each test concentration was prepared in triplicate by adding napropamide directly to nutrient medium of each of the test media.

Test duration: 72 hours.

Observations and sampling: Samples were taken at 24, 48 and 72 hours and the cell densities estimated using a haemacytometer. Analytical samples were taken at the start and end of the exposure period.

Statistical analysis: As described in OECD TG 201 (1984).

Test conditions: 100 ml aliquots of test solution (including algae) was added to triplicate 250 ml conical flasks containing small glass balls, and closed with polyurethane foam bungs. A continuous photoperiod (approximately 3333 lux) was maintained, with a test temperature of 24.3-24.6°C. Inoculated flasks were placed in an orbital shaker.

Food: Nutrient medium.

Results:

Table 9.2.6-3: Inhibition of algal growth following treatment with technical napropamide.

Nominal exposure concentrations (mg as/L)	Mean measured concentrations (mg as/L)	Area under curve at 72 hours (x 10^4 cells/ml)	% of control	Mean growth rate, 0-72 hours (x 10^{-2} cells/ml)	% of control
Control	-	1732	-	6.113	-
4.27	3.36	1666	4	6.127	0
9.39	5.05	1538	11	6.019	2
20.7	6.31	1008	42	5.220	15
45.5	28.3	582.5	66	4.374	28
100	63.7	197.1	89	2.781	55

Growth was high in controls and at the two lowest concentrations and inhibition increased to 55% at the highest concentration tested, 63.7 mg as/L (nominal).

Table 9.2.6-4: Growth inhibition of *Anabaena* by napropamide. Measured concentrations.

Growth Function	Time interval	Estimates EC_{50} values	NOEC

	hours	(mg/L)	(mg/L)
		E_bC_{50}	5.05
Area under growth curve – biomass	0-72	14.2	
		E_rC_{50}	
Average specific growth rate (μ_{ave}/day)	0-72	55.0	

Reviewer's assessment:
In general, the test conditions were in accordance with the requirements of OECD TG 201, Alga, Growth Inhibition Test. The growth rate in the controls met the requirements of the guideline (increase by a factor of 16 during 72 hours). The study is acceptable and the E_rC_{50} = 55.0 mg as/L (measured) can be used for the risk assessment.

A further study to assess the effect of napropamide-M on green algae has been conducted and a summary provide below.

Report

M.K. Kamle (2014b) Alga (*Pseudokirchneriella subcapitata*), growth inhibition test with Napropamide-M technical. UPL Europe Ltd, Unpublished report No.: 501-3-07-6175

Guidelines

OECD guideline 201

GLP

Yes (certified laboratory)

Materials and Methods

Test material	Napropamide-M Technical
Description:	Beige colour crystalline solid
Lot/Batch #:	UPH-08/DNE-263/Tech/20121226.
Purity:	97.26% w/w (total D- + L-isomer)
Stability of test compound:	Stable under test conditions.
Solubility in water:	0.039 g/L at 20^{o}C
Reference substance:	Potassium Dichromate
Test animals	
Species:	*Pseudokirchneriella subcapitata.*
Strain:	ATCC 22662
Source:	American Type Culture Collection, 10801, University of Boulevard, Manassas, Virginia, 20110-2209, USA
Experimental dates:	September 2nd 2013 – September 6th 2013

Test Design

A pre-culture was prepared 3 days prior to commencement of the study and was incubated under the same conditions as those required for the test. The test organisms were used for the definitive test when the population was in the exponential gowth phase. The timing of the pre-culture preparation was intended to provide an adequate number of algal cells required for the experiment (5 x 103 -104 cells/mL).

Exponentially growing cultures of algae (12116 cells/mL) were exposed to nominal test concentrations of 0.8, 1.9, 4.8, 12.0 and 30.0 mg /L. A control with only test medium was also observed. A positive reference of potassium dichromate was tested in an earlier study.

The algae were added to 250 mL conical flasks with 3 replicates per treatment group and 6 replicates in the control. The temperature was measured daily (21-23 °C) and the pH of the test media was measured in each vessel at the start and end of each study (7.08 to 7.99). Continuous illumination was maintained in the range of 5100 – 5233 lux. The cells in the culture flasks were maintained in suspension by agitating the test flasks continuously at 100 ± 2 rpm using an orbital shaker throughout the exposure period.

To determine the algal cell count A volume of 10 mL of the test culture was collected from all replicate flasks at 24, 48 and 72 h. The cell concentration of each replicate was determined once at regular intervals of 24 h using a haemocytometer and microscope.

Analytical verification of the test concentrations was carried out using High Performance Liquid Chromatography in which 25 mL was collected in glass bottles for each test concentration (three replicates of the treatment groups and six replicates of the control group).

As growth inhibition was the most sensitive parameter this was used to determine the NOEC value. The NOEC was calculated using Bartlett's test to check for homogeneity of variance followed by Dunnett's t-test using the individual replicate values of both control and treated groups. The 72 hour EC_{50} values for growth (b), growth rate (r) and yield (γ) with associated 95% fiducial limits were calculated using the Probit analysis method (Finney, 1971).

Results and Discussion

Cell density increased in the control culture by the factor of 64.2 within 72 hours. The co-efficient of variation of average specific growth rates during the whole test period in the control was 0.72%.

The study report states that the Coefficient of Variation of Sectional (Daily) Growth Rate for days 0-1, 1-2 and 2-3 in Control Culture: 2.87, 6.96 and 5.26% respectively.

Analytical results

Analytical verification of the test concentrations was carried out using High Performance Liquid Chromatography.During the main study, the measured values ranged from 89.22 to 94.15% of the nominal concentrations at the test initiation and from 91.19 to 95.13% at the end of the test. All reported results are based on the nominal concentrations of the test item.

Biological Results

The positive control study (JRF Study N° 501-3-07-6266, March 19, 2013 to March 22, 2013) using potassium dichromate as the reference substance determined that the EC_{50} value for inhibition of growth was 1.15 mg/L and 3.77 mg/L for rate reduction.

No significant effects on algal growth parameters were apparent (at 5% by Dunnett's t test with 95% confidence interval) at 0.8 mg/L. This concentration was considered the NOEC in the present study. At 1.9 mg/L a significant impact on algal growth was observed. The effects on growth rate and yield of napropamide-M technical treatments are presented in the following tables.

Table B.9.2.6-1 The percent inhibitions at the tested concentrations in relation to growth inhibition (E_bC_x), growth rate reduction (E_rC_x) and yield inhibition ($E_\gamma C_x$) over the 72 h exposure to napropamide-M Technical

Treatment group (mg test item /L, nominal	Percentage growth (%)		
	Inhibition[1]	Growth rate reduction	Yield inhibition
	(E_bC) 0 – 72 h	(E_rC) 0 – 72 h	($E_\gamma C$) 0 – 72 h
Control (test medium only)	-	-	-
0.8	1.06	0.17	0.65
1.9	17.78*	7.44	27.30
4.8	34.14*	10.55	36.11
12.0	47.98*	14.88	47.20
30.0	93.25	58.82	92.77

1) Area under the growth curve
2) Growth inhibition was the most sensitive parameter for toxicity determination. *=Significant result, Dunnett's t-test p=<0.05

A summary of the calculated effect concentrations is provided in the tables below:

Table B.9.2.6-2 Summary of effect concentrations after time exposure of 72 hours to napropamide-M Technical based on nominal concentrations

E_bC_x values (mg/L) for Growth Inhibition		95% Fiducial Limits ~(mg/L)		Regression Equation (y = a + bx
		Lower Limit	Upper Limit	
E_bC_{10}	1.94	0.95	3.95	y = -3.29 + 2.13x
E_bC_{20}	3.12	1.78	5.48	y = -3.29 + 2.13x
E_bC_{50}	7.74	4.91	12.20	y = -3.29 + 2.13x

E_rC_x values (mg/L) for Growth Rate Reduction		95% Fiducial Limits ~(mg/L)		Regression Equation (y = a + bx
		Lower Limit	Upper Limit	
E_rC_{10}	4.81	2.19	10.55	y = -2.42 + 1.67x
E_rC_{20}	8.82	4.83	16.11	y = -2.42 + 1.67x
E_rC_{50}	28.18	12.71	62.51	y = -2.42 + 1.67x

E_yC_x values (mg/L) for yield inhibition		95% Fiducial Limits ~(mg/L)		Regression Equation (y = a + bx
		Lower Limit	Upper Limit	
$E_\gamma C_{10}$	1.90	0.91	3.99	y = -3.30 + 2.14 x
$E_\gamma C_{20}$	3.06	1.66	5.61	y = -3.30 + 2.14 x
$E_\gamma C_{50}$	7.56	4.38	13.03	y = -3.30 + 2.14 x

Conclusions

No statistically significant effects on growth parameters of *Pseudokirchneriella subcapitata* were observed at the concentration of 0.8 mg napropamide-M technical/L during the test period of 72 hours. At concentration of 1.9 mg test item/L, napropamide-M Technical exhibited significant impact on algal growth parameters. This was the lowest concentration exhibiting significant impact on algal growth parameters.

As growth inhibition was the most sensitive parameter for toxicity determination, this parameter was used to determine the NOEC which was 0.8 mg/L.

The 72 h EC_{50} values determined were 7.74 mg/L, 28.18 mg/L and 7.56 mg/L for growth inhibition (E_bC_{50}), growth rate reduction (E_rC_{50}) and yield inhibition ($E_\gamma C_{50}$), respectively.

RMS Comments

The study report states that all of the validity criteria have been met. This has been reconsidered by the RMS, the biomass in the control increased by a factor of 64.2 and the coefficient of variation of average growth between control replicates was 0.36%, both of which pass the validity criteria of >16 and <7% respectively. However the coefficient of variation of sectional (daily) growth rate for days 0-1, 1-2 and 2-3 in the control culture has been calculated incorrectly (calculating the coefficient of variation across the individual replicates for each of the time periods rather than across the time periods for each replicate), stating values of 2.87, 6.96 and 5.26% respectively. This has been re-calculated by the RMS and a value of 69.2% was determined, which fails the criteria of <35%. Based on this, this study is not considered suitable for use in the risk assessment.

B.9.2.7. Effects on aquatic macrophytes

In accordance with the data requirements under Regulation (EU) No. 283/2013, effects on aquatic macrophytes are required for substances with herbicidal activity. Studies have therefore been conducted on *Lemna gibba* and *Myriophyllum spicatum* with napropamide-M. As *Lemna gibba* was shown to be the more sensitive species, studies have been conducted with the metabolites Isomer I and Isomer II. These studies are summarised below.

Report

C. Ramsden (2015b) Assessment of the effect of napropamide-M technical (HBW07) on *Lemna*, growth inhibition test. UPL Europe Ltd, Unpublished report No.: ENV-13-046. Including Amendment No.1.

Guidelines

OECD guideline 221

GLP

Yes (certified)

Materials and Methods

Test material	Napropamide-M Technical
Description:	Orange-white crystals
Lot/Batch #:	UPH-08/DNE-263/Tech/20121226.
Purity:	97.26 % w/w (total D- + L-isomer)
Stability of test compound:	Not stable over time.
Solubility in water:	0.039 g/L at 20°C.
Reference substance:	Not stated.
Test animals	
Species:	*Lemna gibba*.
Strain:	Not stated.
Source:	Canadian Phycological Culture Centre (CPCC), University of Waterloo, Canada.
Experimental dates:	25th of November 2013 – 4th of December 2013

Study Design

The test was conducted using *Lemna gibba* taken from an on-site culture which was maintained in conditions similar to those used for the difinitive test. Colonies used for the study were taken from cultures in the exponential growth phase.

For the definitive test *Lemna gibba* were exposed to five concentrations of the test item and control group under semi-static conditions. A range finding test was conducted to determine the sensitivity of the test system and as a result of this the following concentrations were tested; 0.021, 0.062,0.187,0.562, 1.686 mg/L. Three replicates of each concentration were used along and six for the control. Test item solutions were freshly prepared at days 0, 2 and 4 by dilution of a stock filtrate solution using *Lemna* medium. This was achieved by dissolving 50.25 mg of the test item in 500 mL of medium and placing it on a magnetic stirrer at 4,500 rpm for 3 hours. The resulting solution/suspension mixture was then filtered through a grade 1 Watman filter paper.

Test vessels consisted of glass jars with a capacity of 300 mL and contained 120 mL of solution. Following this 3 colonies comprising of 9 fronds were added to each vessel. At renewal (on days 2 and 4) new vessels were set up with fresh media and the *Lemna gibba* transferred from the previous vessels into the new vessels.

On days 0, 2, 4 and 7 (0, 48, 96 and 168 hours ± 1 hour) frond numbers were counted manually and recorded. Changes in plant development e.g. frond size, appearance, indication of necrosis, chlorosis, gibbosity, colony break up or loss of buoyancy and changes in root length or appearance were noted.

The pH (7.51-9.31) was determined in each of the test and control bulk solutions at the start of each renewal period and in a single vessel at each concentration at the end of each renewal period. Temperature was in the range of 22 – 25^{o}C and was recorded in a surrogate vessel containing *Lemna* media for the duration of the test. Continuous illumination was used and measured on day 0 (8020 lux) and day 7 (7760 lux).

Three samples of the *Lemna* culture representative of that used in the test were taken at the set-up of the test to give a dry weight to be used in the calculation of yield. This was done by collecting 30 colonies of three fronds (90 fronds in total per replicate, three replicates in total). The *Lemna* were not rinsed or blotted dry. They were then placed in pre-weighed glass vials and dried at approximately 60 °C to a constant weight. The dry weights were recorded and the mean dry weight for 9 fronds was calculated.

The concentrations of all the test item solutions were determined at the beginning and end of each renewal period. The samples were analysed using an external standard high performance liquid chromatography (HPLC) method. This was conducted in house.

To determine the frond count NOEC, ECx values the results were compared statistically using Wilcoxon/Bonferroni test to the control group using to the level of 0.05. Nonlinear regression analysis was conducted, the model used for specific growth rate was a 4 parameter log-logistic and the model used for yield was a 4 parameter cumulative log-normal. For the dry weight EC_X values the results were also compared statistically to the control group using Dunnett's test, significance to the level of 0.05 was used. It was not possible to generate NOEC values for the dry weight endpoints as there were effects observed at all concentrations including the lowest tested concentration.

Results and Discussion

The study met the validity criteria of the guideline as doubling time of frond number in the control was less than 2.5 days (1.44 days) and the average specific growth rate in the control was greater than 0.275 d^{-1} (0.481 $^{d-1}$).

Analytical results

The samples were analysed using an external standard high performance liquid chromatography (HPLC) method . The results showed that the test item was not stable over time, based on this it was necessary to use a semi-static regime (Table B.9.2.7-1). The geometric mean measured values for test item concentrations in the exposure groups were used for all data analysis.

Table B.9.2.7-1: Summary table detailing geometric mean test item concentrations and percentage recovery based on HPLC analysis.

Nominal Concentration (mg a.s./L)	Geometric mean test item concentration (mg a.s.L $^{-1}$)	Recovery (% of Nominal)
Control	-	-
0.021	0.016	77
0.062	0.039	62
0.187	0.104	56
0.562	0.313	56
1.686	0.874	52

Biological results

This test shows that in terms of frond count, napropamide-M technical has an E_rC_{50} of 0.07791 mg/L^{-1} and an E_yC_{50} of 0.05399 mg/ L^{-1}. The frond count NOEC value for both endpoints was 0.016 mg/ L^{-1}. There was a significant difference compared to the control in growth rate and yield at all concentrations above 0.016 mg/L.

Table B.9.2.7-2 Mean frond numbers, mean %Ir and mean %Iy

Geometric mean test item concentration (mg L-1)	Mean frond numbers				%Ir*	%Iy*
	0 days	2 days	4 days	7 days		
Control (Media)	9	23 (±2)	58 (±5)	260 (±20)	N/A	N/A
0.016	9	20 (±2)	48 (±6)	277 (±14)	-1.95	-6.82
0.039	9	18 (±1)	36 (±3)	202 (±31)	7.74 *	23.15 *
0.104	9	16 (±2)	26 (±3)	68 (±8)	39.88*	76.41 *
0.313	9	13 (±1)	20 (±2)	44 (±4)	53.06 *	86.16 *
0.874	9	13 (±1)	16 (±1)	32 (±2)	62.52 *	90.94 *

*=significant result, Wilcoxon/Bonferroni Test $p<0.05$

The secondary measurement of dry weight showed that napropamide-M technical has an E_rC_{50} of >0.874 mg/L^{-1} and an E_yC_{50} of 0.01941 mg/L^{-1}. It was not possible to generate NOEC values for either endpoint as there were effects observed at all concentrations including the lowest tested concentration.

Table B.9.2.7-3 Mean dry weights (mg, total Lemna mass for each group), mean %Ir and mean %Iy

Geometric mean test item concentration (mg L-1)	Mean dry weights		%Ir	%Iy
	0 days	7 days		
Control (Media)	1.00	27.61 (±0.0)	N/A	N/A
0.016	1.00	18.76 (±4.6)	11.51 *	33.3 *
0.039	1.00	13.26 (±4.9)	22.03 *	53.9 *
0.104	1.00	9.33 (±3.1)	32.63 *	68.7 *
0.313	1.00	8.13 (±3.3)	36.86 *	73.2 *
0.874	1.00	7.44 (±3.2)	39.52 *	75.8 *

*=significant result, Dunnett's test $p<0.05$

Table B.9.2.7-4 Frond count EC_{10}, EC_{20} and EC_{50} values for growth rate and yield

	mg test item L^{-1}	95% confidence limits	
		Lower	Upper
Growth rate			
E_rC_{10}	0.03072	N/A	0.04379
E_rC_{20}	0.04331	0.02497	0.05841
E_rC_{50}	0.07791	0.06191	0.09804
NOEC	0.016	-	-
Yeild			
E_yC_{10}	0.02623	0.01621	0.03249
E_yC_{20}	0.03361	0.02641	0.04004
E_yC_{50}	0.05399	0.04623	0.06306
NOEC	0.016	-	-

Table B.9.2.7-5 Dry weight EC_{10}, EC_{20} and EC_{50} values for growth rate and yield

	mg test item L^{-1}	95% confidence limits	
		Lower	Upper
Growth rate			
E_rC_{10}	0.003003	0.000130	0.01629
E_rC_{20}	0.02831	0.008923	0.07449
E_rC_{50}	> 0.874	N/A	N/A
Yeild			
E_yC_{10}	0.003588	0.002917	0.004253
E_yC_{20}	0.00669	0.005921	0.00748
E_yC_{50}	0.01941	0.01856	0.02031

It was not possible to generate NOEC values for napropamide-M technical for the dry weight endpoints as there were effects observed at all concentrations including the lowest tested concentration.

Conclusions

This test shows that in terms of frond count, napropamide-M technical has an E_rC_{50} of 0.07791 mg/L-1 and an E_yC50 of 0.05399 mg/ L-1. The frond count NOEC value for both endpoints was 0.016 mg/ L-1. The secondary measurement of dry weight showed that napropamide-M technical has an E_rC_{50} of >0.874 mg/L-1 and an E_yC50 of 0.01941 mg/L-1. It was not possible to generate NOEC values for either endpoint as there were effects observed at all concentrations including the lowest tested concentration.

RMS Comments

It is noted that the pH varied by more than 1.5 units in the control (1.63 units) between days 4 and 7. As the validity criteria were met and the control showed good development this is considered to be a minor deviation.

As it was not possible to generate NOEC values based on dry weight due to effects being observed for dry weight at all of the concentrations tested, the EC_{10} values will be used in place of the NOEC. This study is considered suitable for use in the risk assessment.

This study was conducted in line with OECD 221, under GLP compliance; all validity criteria were met. The RMS considers that this study is suitable for use in the risk assessment with a E_rC_{50} of 0.08 mg a.s./L.

Report

D. Juckeland (2012a) Effects of napropamide metabolite Isomer I on *Lemna minor* in a growth inhibition test under semi-static test conditions. UPL Europe Ltd, Unpublished report No.: 11 10 48 017 W

Guidelines

OECD Guideline 221 (March 2006)

GLP

Yes (certified)

Materials

Test material	Napropamide metabolite Isomer I
Description:	Not stated.
Lot/Batch #:	UPV/866-29/ISM-I/16
Purity:	97.69 % w/w
Stability of test compound:	Stable under normal conditions
Solubility in water:	0.039 g/L at 20°C.
Reference substance:	3,5-dichlorophenol.
Test animals	
Species:	*Lemna minor*.
Strain:	Not stated.
Source:	Insititut für allgemein Botanik' at the University of Jena, Germany.
Experimental dates:	19th of September 2011 – 27th of September 2011.

Methodology

The *Lemna* used in the study was cultured in a freshwater algal medium under similar conditions as those used for the definitive test. Colonies used in the test were transferred into fresh sterile medium 7 days before test initiation and acclimatised to test conditions.

Glass beakers with 100 mL of solution were used as the test vessels. Initial colonies consisted of 2- 4 fronds of a similar size. Test concentrations of 0.042, 0.189, 0.679, 1.96 and 6.84 mg a.s/L (mean measured concentrations 0.006, 0.088, 0.518, 1.70, 5.81 mg a.s/L) were used in the definitive test under a semi-static test regime with renewal of solutions on days 2 and 4. A reference test was also conducted with 3,5-dichlorophenol at an earlier date.

For each test concentration three test replicates with one additional vessel for analysis and were used along with six for the control. The test vessels were kept in a temperature controlled climatic chamber (23.1 - 25.8 ºC) with an average constant illumination of 8000 lx (differences from the average light intensity over the test area did not exceed the range ± 15 % and a pH in the control of 5.52-6.69. At test start each test vessel was filled with 100 mL of the appropriate test solution with 9 *Lemna* fronds.

On days 0, 2 4 and 7 the number of fronds were counted and any observations in the plant development e.g. appearance, necrosis etc. were recorded. The mean final plant dry weight for all replicates was recorded on day 7.

For the analytical verification of the test concentrations solutions were sampled in duplicate directly after preparation of the test solutions at test initiation, before and after renewal on days 2 and 4 and also at the end of the test at day 7. MS detection was used to quantitate the specimens of treatments 1, 2 and 3; for treatment 4 the mean of UV and MS detection was used; the quantitation of the treatments 5 and 6 was based solely on UVdetection.

The seven day EC_{50} value with 95% confidence limits was calculated by Probit analysis (Finney, 1971). For estimation of the NOEC it was necessary to compare treatments using analysis of variance (ANOVA) techniques. The values for all replicates of each concentration were compared with the untreated control values using an appropriate multiple comparison method (Williams t-test for homogeneous variances, $p \leq 0.05$). As a test for homogeneity of variances Levene´s Test was used.

Results and discussion

All validity criteria have been met. The measured doubling time of the frond number in the untreated control was on average <2.5 days (actual result 2.2 days).This corresponded to a 9-fold increase in frond number over the 7-day study period which was above the criteria of >7 fold. The average specific growth rate in the control was 0.313 d^{-1} for frond number which was also above the criteria of >0.275 d^{-1}.

Analytical results

The results of the analysis of the test concentrations showed that the percentage recovery was not within 20% of the initial measured concentrations (Table B.9.2.7-6). The initial measured concentrations were 0.042, 0.189, 0.679, 1.96 and 6.84 mg a.s/L and the mean measured concentrations were 0.006, 0.088, 0.518, 1.70 and 5.81 mg a.s/L. The endpoints will therefore be based on the mean measured concentrations.

Table B.9.2.7-6: Summary table detailing mean measured test item concentrations and percentage recovery based on MS and UV detection analysis.

Initial measured concentration (mg a.s./L)	Mean measured concentration (mg a.s.L^{-1})	Recovery (% of initial)
Control	-	-
0.042	0.006	59.5
0.189	0.088	65.8
0.679	0.518	80.3
1.96	1.70	87.8
6.84	5.81	86.2

Biological results

The results of a separate routine *Lemna* growth inhibition reference toxicity test (performed by BioChem agrar) using 3,5-dichlorophenol are reported to verify the sensitivity of the test system. The ErC_{50} (frond number) value was 2.40 mg /L. This value is within the range 2.2 - 3.8 mg/L, demonstrating that the test system was suitably sensitive.

At the mean measured concentrations of 0.006, 0.088, 0.518, 1.70 and 5.81 mg a.s/L, the growth rate was inhibited by 0.0, 3.2, 7.7, 23.3 and 42.2 % for frond number and by 3.4, 9.0, 24.2, 33.7 and 41.6

% for biomass respectively compared to the control. A multiple comparison procedure (Williams t-test, p <0.05) revealed statistically significant differences with respect to growth rate at test concentrations ≥0.088 mg a.s/L for frond number and biomass.

Biomass increase was inhibited by 9.1, 22.0, 49.6, 62.0 and 70.1 % at the initial mean measured concentration of 0.006, 0.088, 0.518, 1.70 and 5.81 mg a.s/L respectively compared to the untreated control. A multiple comparison procedure (Welch -t-test p < 0.05) revealed statistically significant differences with respect to yield (biomass increase) at concentrations of ≥0.088 mg a.s/L.

On day 4 chlorotic effects were observed at concentrations ≥ 1.70 mg a.s/L. On day 7 effects were observed at concentrations ≥0.518 mg a.s/L. On days 2, 4 and 7 a decreased root growth was observed at test concentrations ≥0.088 mg a.s/L.

Table B.9.2.7-7 Effects of Napropamide Metabolite Isomer - I on growth rate and yield of *Lemna minor*

Treatment group mg a.s./L, mean measured	Final frond number replicate mean day 7	Biomass (dry weight) replicate mean day 7 (mg)	% Inhibition			
			Average specific growth rate (% Ir)		Yield (% Iy)	
			frond number	biomass	frond number	biomass
Control	80.5(±2.81)	16.6	-	-	-	-
0.006	82.7(±3.79)	15.2	0.0	3.4	0.0 (-3.0)	9.1
0.088	75.0(±2.65)	13.3	3.2 +	9.0 +	7.7 +	22.0 +
0.518	68.0(±4.36)	9.1	7.7 +	24.4+	17.5 +	49.6 +
1.70	48.3(±2.08)	7.2	23.3 +	33.7+	45.0 +	62.0 +
5.81	32.0(±2.00)	5.9	42. 2+	41.6+	67.8 +	70.1 +

+ statistically significantly different to the untreated control (Williams t-test; Welch-t-test; p <0.05, one-sided)

In the case of the growth rate the E_rC_{50} values were higher than the highest test concentration of 5.81 mg a.s/L and so the E_rC_{50} values were considered to be >5.81 mg/L. The effect concentrations are shown in the table below.

Table B.9.2.7-8 Effect concentrations of the test item Isomer 1: summary of statistical analysis (based on initial mean measured and mean measured concentrations)

Effect concentration mg/L (95 % C.I)	Average specific growth rate		Yield	
	Frond number	Biomass	Frond number	Biomass
Mean measured	E_rC_{10} = 0.560 (0.330 - 0.796)	E_rC_{10} = 0.060 (0.011 - 0.149)	E_yC_{10} = 0.214 (0.075 - 0.383)	E_yC_{10} = 0.010 (0.001 - 0.033)
Mean measured	E_rC_{20} = 1.44* (1.07 - 1.80)	E_rC_{20} = 0.365 (0.146 - 0.639)	E_yC_{50} = 2.38 (1.74 - 3.41)	E_yC_{50} = 0.729 (0.395 - 1.37)

* EC_{50} values could not be calculated as effects of >50 % were not seen at the highest test concentration, therefore EC_{20} values are shown

Conclusion

For growth rate inhibition 50 % effects were not seen at the highest test concentration and so the E_rC_{50} was considered to be >5.81 mg a.s/L. For yield inhibition the E_yC_{50} was calculated to be 2.38 mg a.s/L based on frond number and 0.729 mg a.s/L based on biomass. The NOEC for all parameters was calculated to be 0.006 mg a.s/L, all endpoints are based on mean measured concentrations.

RMS Comments

This study was conducted in line with OECD 221, under GLP compliance; all validity criteria were met. It is noted that raw data for dry weight is not reported in the study report, therefore there is some uncertainty present in this area, as this is not the primary measurement the RMS considers that the outcome of the study can still be relied upon. The RMS considers that this study is suitable for use in the risk assessment with an ErC50 of >5.81 mg a.s. /L.

Report

D. Juckeland (2012b) Effects of napropamide metabolite Isomer II on *Lemna minor* in a growth inhibition test under semi-static test conditions. UPL Europe Ltd, Unpublished report No.: 11 10 48 018 W

Guidelines

OECD Guideline 221 (March 2006)

GLP

Yes (certified)

Materials

Test material	Napropamide metabolite Isomer II
Description:	Brown solid.
Lot/Batch #:	UPV/886-199/ISM-II/20
Purity:	97.07 % w/w
Stability of test compound:	Stable under normal conditions
Solubility in water:	0.039 g/L at 20^{o}C.
Reference substance:	3,5-dichlorophenol.
Test animals	
Species:	*Lemna minor*.
Strain:	Not stated.
Source:	Insititut für allgemein Botanik' at the University of Jena, Germany.
Test dates:	2nd of September 2011 – 12th of September 2011.

Test Method

The *Lemna* used in the study was cultured in a freshwater algal medium under similar conditions as those used for the definitive test. Colonies used in the test were transferred into fresh sterile medium 7 days before test initiation and acclimatised to test conditions.

Glass beakers with 100 mL of solution were used as the test vessels. Initial colonies consisted of 2- 4 fronds of a similar size. Test concentrations of 0.123, 0.241, 0.332, 0.515 and 0.741 mg a.s/L (mean measured concentrations were 0.023, 0.038, 0.110, 0.208 and 0.321 mg a.s/L) were used in the definitive test under a semi-static test regime with renewal of solutions on days 2 and 4. A reference test was also conducted with 3,5-dichlorophenol at an earlier date.

For each test concentration and control 3 test replicates with one additional vessel for analysis and were used. The test vessels were kept in a temperature controlled climatic (22.8 - 22.9 ºC) with an average constant illumination of 8000 lx measured at a spectral range of 400-700 nm (differences from the average light intensity over the test area did not exceed the range ± 15 % and a pH in the control of 3.46-6.50. At test start each test vessel was filled with 100 mL of the appropriate test solution with 9 *Lemna* fronds.

On days 0, 3 3 and 7 the number of fronds were counted and any observations in the plant development e.g. appearance, necrosis etc. were recorded. The mean final plant dry weight for all replicates was recorded on day 7.

For the analytical verification of the test concentrations solutions were sampled in duplicate directly after preparation of the test solutions at test initiation, before and after renewal on days 2 and 4 and also at the end of the test at day 7. The samples were analysed by high performance liquid chromatography in accordance with a previously validated analytical method.

The seven day EC_{50} value with 95% confidence limits was calculated by Probit analysis (Finney, 1971). For estimation of the NOEC it was necessary to compare treatments using analysis of variance (ANOVA) techniques. The values for all replicates of each concentration were compared with the untreated control values using an appropriate multiple comparison method (Williams t-test for homogeneous variances, $p \leq 0.05$). As a test for homogeneity of variances Levene´s Test was used.

Results and discussion

All validity criteria were met. The measured doubling time of the frond number in the untreated control was on average 2.24 days meeting the criteria of <2.5 days. This corresponded to a 9-fold increase in frond number over the 7-day study period which meets the criteria of >7 fold increase. The average specific growth rate in the control was 0.31 d^{-1} for frond number meeting the criteria of >0.275 d^{-1}.

Analytical results

The samples were analysed by high performance liquid chromatography to determine if the test concentrations remained constant. The results showed that the concentration did not stay within 20% of the the initialy measured values (B.9.2.7-9) therefore the endpoints will be based on the mean measured concentrations.

Table B.9.2.7-9: Summary table detailing mean measured test item concentrations and percentage recovery based on MS and UV detection analysis.

Initial measured concentration (mg a.s./L)	Mean measured concentration (mg a.s. L^{-1})	Recovery (% of initial)
Control	-	-
0.123	0.023	57.05
0.241	0.038	55.19
0.332	0.110	58.46
0.515	0.208	60.49
0.741	0.321	61.81

Biological results

The results of a separate routine *Lemna* growth inhibition reference toxicity test using 3,5-dichlorophenol are reported to verify the sensitivity of the test system. The ErC_{50} (frond number) was 2.40 mg /L. This value is within the expected range of 2.2 - 3.8 mg/L.

At the mean measured concentrations of 0.023, 0.038, 0.110, 0.208 and 0.321 mg a.s/L the growth rate was inhibited by 1.4, 3.0, 17.1, 23.8, 31.5 % for frond number and by 2.2, 0.1, 7.5, 6.4 and 11.9 % for biomass respectively compared to the control. Statistically significant differences with respect to growth rate at test concentrations ≥0.110 mg a.s/L for frond number and biomass were determined.

Yield for frond number was inhibited by 3.2, 7.2, 34.8, 45.2 and 55.6% and biomass increase was inhibited by 6.2, 0.5, 21.1, 18.4 and 30.6% at the mean measured concentrations of 0.023, 0.038, 0.110, 0.208 and 0.321 mg a.s/L respectively compared to the untreated control. Significant

differences with respect to yield (frond number) at test concentrations ≥0.038 mg a.s/L was determined.

Table B.9.2.7-10 Effects of Napropamide Metabolite Isomer - II on growth rate and yield of *Lemna minor*

Treatment group mg a.s./L, mean measured	Final frond number replicate mean day 7	Biomass (dry weight) replicate mean day 7 (mg)	% Inhibition			
			Average specific growth rate (% Ir)		Yield (% Iy)	
			frond number	biomass	frond number	biomass
Control	76.5(±3.78)	14.2	-	-	-	-
0.023	74.3(±6.11)	14.7	1.4	2.2	3.2	6.2
0.038	71.7(±0.58)	15.6	3.0	0.1	7.2+	0.5
0.110	53.0(±1.00)	11.4	17.1 +	7.5 +	34.8 +	21.1 +
0.208	46.0(±3.61)	11.7	23.8 +	6.4 +	45.2 +	18.4 +
0.321	39.0(±2.65)	10.1	31.5 +	11.9 +	55.6 +	30.6 +

+ statistically significantly different to the untreated control (Williams t-test; $p < 0.05$, one-sided)

On days 5 and 7 chlorotic effects were observed at concentrations ≥ 0.208 mg a.s/L. On days 5 and 7 a decreased root growth was observed at test concentrations ≥0.038 mg a.s/L.

Table B.9.2.7-11 Effect concentrations of the test item Isomer II: summary of statistical analysis (based on initial mean measured and mean measured concentrations)

Effect concentration mg/L (95 % C.I)	Average specific growth rate		Yield	
	Frond number	Biomass	Frond number	Biomass
Mean measured	E_rC_{10} = 0.072 (0.055 - 0.181)	E_rC_{10} = 0.268 (0.177 - 0.627)	E_yC_{10} = 0.035 (0.024 - 0.046)	E_yC_{10} = 0.066 (0.008 - 0.113)
Mean measured	E_rC_{20} = 0.161* (0.141 - 0.181)	E_rC_{20} = >0.321*	E_yC_{50} = 0.244 (0.213 - 0.286)	E_yC_{20} = 0.169* (0.088 - 0.270)

* EC_{50} values could not be calculated as effects of >50 % were not seen at the highest test concentration, therefore EC_{20} values are shown

Conclusion

For growth rate inhibition 50 % effects were not seen at the highest test concentration and so the E_rC_{50} was considered to be >0.321 mg a.s/L. For yield inhibition the E_yC_{50} was calculated to be 0.244 mg a.s/L based on frond number and 0.169 mg a.s/L based on biomass. The NOEC for all growth rate was calculated to be 0.332 mg a.s/L based on mean measured concentration.

RMS Comments

It is noted that the pH varied by more than 1.5 units (3.46-6.50) howvever this was only on one occasion. As the validity criteria have been met and the control plants appered to be healthy, this is considered to be a minor deviation It is also noted that raw data for dry weight is not reported in the study report, therefore there is some uncertainty present in this area, as this is not the primary measurement the RMS considers that the outcome of the study can still be relied upon.

The NOEC proposed by the applicant (0.332 mg a.s/L) resulted in a 31.5% reduction in frond number and an 11.5% reduction in biomass therefore the RMS has determined that a more appropriate NOEC is 0.038 mg a.s/L for which there is a 3% reduction in frond number and an 0.1% reduction in biomass.With this amendment this study is considered suitable for use in the risk assessment.

This study was conducted in line with OECD 221, under GLP compliance; all validity criteria were met. The RMS considers that this study is suitable for use in the risk assessment with a E_rC_{50} of >0.321 mg a.s./L.

Report

H. Hermes (2015) Napropamide-M HBW07: Toxicity to the aquatic plant *Myriophyllum spicatum* in a semi static growth inhibition test with a prior rooting phase. UPL Europe Ltd, Unpublished report No.: 98011215

Guidelines

OECD Guideline 239 (2014) (Deviations: None)

GLP

Yes (certified laboratory)

Test material

Test material	Napropamide-M Technical
Description:	Off-white crystals
Lot/Batch #:	UPV/714-181/DEV/014.
Purity:	97.2% w/w (total D- + L-isomer)
Stability of test compound:	Not stable over time.
Solubility in water:	0.039 g/L at 20^{o}C.
Reference substance:	Non stated.
Test animals	
Species:	*Myriophyllum spicatum.*
Strain:	Not stated.
Source:	The sterile plants introduced in the test were taken from ibacon's in-house laboratory culture.
Experimental dates:	26th of February 2015 – 4th of March 2015.

Test system

Plants used in the study were taken from the laboratories in house culture. The plants of the stock culture were maintained under similar conditions as those in the definitive test. The side shoots used for the definitive test had a length of 6 ± 1 cm before introducing them into the test.

The test sediment consisted of 5% sphagnum moss peat, 74.8 % quartz sand, 20.0 % kaolin clay. CaCO3 of chemically pure quality was added to adjust the pH of the final mixture of the sediment to 6.9. Small plastic plant pots (approx. 8.5 cm diameter, 7 cm high and with a volume of approx. 400 mL) were used as containers for potting the plants into the sediment. Test beakers of 2000 mL volume with approximately 1800 mL test medium were used to provide a minimum overlaying water depth of 12 cm. Five shoot tips were planted into each pot containing the sediment with two nodes covered into the sediment. In order to induce root development they were kept prior to the test start for 7 days in test water.

After the pre-culture period, on Day 0 of the exposure period, two of the five plants in each test beaker were removed to leave three individual plants of similar size and appearance and test item added to the respective replicates.

The exposure was conducted under semi-static conditions with six test concentrations; 0.048, 0.108, 0.287, 1.07, 4.86 and 23.3 mg a.s/L and a control, four replicates were used for each treatment concentration and six for the control. In addition a replicate of the highest test concentration was prepared without plants or sediment (referred to as abiotic control). At each renewal period (days 4,7,11 and 14) the aged water was removed completely and replaced with fresh media.

At the start of the exposure period (Day 0) the shoot length above the sediment and the length of any side shoots was measured. To determine the wet and dry weight after 14 days exposure, plants were

weighed after blotting to remove any water and then then dry weight determined by drying at 60 ºC for over 2 hours. Any sublethal symptoms e.g. growth abnormalities, chlorosis or necrosis were recorded at each test medium renewal and at the end of the test.

The test was carried out in a controlled environment room, the temperature was measured daily in a test vessel filled with water and incubated under the same conditions as the test vessels (20 -22^{o}C). The pH-values were measured in all freshly prepared and aged test media of all test concentrations and the control at the start and end of each renewal period (8.0 – 10.3). The oxygen-values were measured in all freshly prepared and aged test media of all test concentrations and the control at the start and end of each renewal period (4.8 – 16.8 mg/L). Illumination was achieved by fluorescent tubes, installed above the test vessels. Measurements were performed at test start at 6 places distributed over the experimental area at the surface of the test media. Differences in light intensity over the test area did not exceed ± 15 % (8380 – 9990 lux).

The samples collected at the start of the experimental period and after 4, 7, 11 and 14 days were analysed via HPLC-method with UV detection (HPLC-UV). The EC_{50} values and their 95 %-confidence limits were calculated by Probit analysis. For the determination of the 14-day NOEC, the calculated growth rates and yields were tested for significant differences compared to the control values by Dunnett's t-test. The statistical evaluations were applied to the mean values per replicate. The software used to perform the statistical analysis was ToxRat Professional, Version 3.0.0, ToxRat Solutions GmbH.

Results

All of the validity criteria were met. The mean coefficient of variation for yield wet weight was 10.9%, the mean total shoot length and total fresh weight in the control more than doubled during the exposure period of 14 days as there was a 4.5 increase in total shoot length and a 5.4 increase in fresh weight.

Analytical results

The results of the HPLC-UV analysis showed that concentration of the test item did not remain within 80 -120% of nominal therefore the endpoints are based on the time weighted mean. The summary of the analytical results is displayed in table B.9.2.7-12.

Table B.9.2.7-12 Summary of analytical results

Sample description	Initial mean (mg a.s./L)	Time weighted mean (mg a.s/L)	% Recovery
Control	n/a	n/a	n/a
1:243 Dilution of filtrate	0.148	0.048	32.4
1:81 Dilution of filtrate	0.443	0.108	24.4
1:27 Dilution of filtrate	1.203	0.287	23.9
1:9 Dilution of filtrate	3.854	1.07	27.8
1:3 Dilution of filtrate	11.685	4.86	41.6
Filtrate	35.735	23.3	65.2

Biological results

With the treatment groups there were significant reductions in yield at 23.3, 4.86 and 1.07 mg a.s/L after 14 days compared to the control. Statistically significant inhibitions for growth rate were determined at 23.3, 4.86 and 1.07 mg a.s/L.

Table B.9.2.7-13: % inhibition of yield and growth rate based on shoot length, wet weight and dry weight of *Myriophyllum spicatum*after after14 days of exposure to Napropamie-M at various concentrations.

Concentration	Shoot Length	Wet Weight	Dry Weight

of Napropimide-M (mg a.s./L	% inhibition of yield	% inhibition of growth rate	% inhibition of yield	% inhibition of growth rate	% inhibition of yield	% inhibition of growth rate
Control	-	-	-	-	-	-
0.048	4.6	4.5	-12.7	-5.8	3.1	1.2
0.108	-3.3	-7.9	-1.5	-0.8	7.4	3.6
0.287	8.6	1.1	20.8*	11.1	15.8	8.1
1.07	47.4*	27.5*	55.9*	38.4*	36.6*	23.2*
4.86	88.0*	75.2*	82.5*	67.4*	74.2*	58.6*
23.3	90.98	81.4*	83.3*	67.7*	71.8*	54.1*

*=Significant result, Dunnett's t-test, $p<0.05$

The appearance of the plants at 0.048 and 0.108 mg a.s/L were comparable to the controls. At the higher concentrations the plants showed some sublethal effects compared to the control. At 23.3 mg a.s/L and 4.86 mg a.s/L necrosis and shortened shoot tips were observed, chlorosis and shortened shoot tips were observed at 1.07 mg a.s/L and slight chlorosis at 0.287 mg a.s/L (table B.9.2.7-14).

Table B.9.2.7-14 Sub-lethal effects on *Myriophyllum spicatum*after after exposure to Napropamie-M at various concentrations.

Concentration of Napropimide-M (mg a.s./L	Sublethal effects during the test		
	Day 0	Day 7	Day 14
Control	0	0	0
0.048	0	0	0
0.108	0	3	0
0.287	0	3	(2)
1.07	0	(2), 3	2, 3
4.86	0	2	(1), 3
23.3	0	1	1, 3

Key: 0 = No effect, 1 = Necrosis, 2 = Chlorosis, 3 = Shortened shoot tips, () = Only slightly pronounced.

Table B.9.2.7-15 Influence of napropamie-M on the growth of *Myriophyllum spicatum*

	Yield (total shoot length) mg a.s/L (95 % C.I)	Growth rate (total shoot length) mg a.s/L (95 % C.I)	Yield (wet weight) mg a.s/L (95 % C.I)	Growth rate (wet weight) mg a.s/L (95 % C.I)	Yield (dry weight) mg a.s/L (95 % C.I)	Growth rate (dry weight) mg a.s/L (95 % C.I)
EC_{50}	1.21 (0.621-2.34)	2.35 (1.08-5.16)	0.904 (0.292-2.81)	2.58 (0.616-10.8)	2.10 (0.40-11.2)	6.99 (0.918-50.1)
EC_{20}	0.467 (0.274-0.806)	0.702 (0.369-1.34)	0.237 (0.094-0.610)	0.328 (0.102-1.08)	0.280 (0.072-1.12)	0.522 (0.107-2.63)
EC_{10}	0.284 (0.163-0.496)	0.373 (0.192-0.727)	0.117 (0.044-0.313)	0.112 (0.033-0.385)	0.097 (0.023-0.413)	0.134 (0.026-0.691)
NOEC	0.287	0.287	0.108	0.287	0.287	0.287

Conclusion

The influence of napropamide-M on the growth of the *Myriophyllum spicatum* was assessed in a semi static dose response test. The 14 day NOEC was determined to be 0.287 mg a.s/L and based on growth rate the EC_{50} values were determined to be 2.35, 2.58 and 6.99 mg a.s/L for shoot length, wet weight and dry weight.

RMS Comments

It is noted that the pH was out of range in the control, (8.0 -10.3) varying by more than 1.5 units, it is also noted that there was a separation factor of more than 3.2 between the test concentrations. As the validity criteria have been met and the control plants appered to be healthy, these devations are considered to be minor. With reference to section B.5.1.2.2 , methods of analysis, Volume CA B.5, the Linear range tested = 0.025 – 9.93 mg/L, this is not ideal however as the endpoints obtained in the study fell within this range, this is considere to be acceptable.

This study was conducted in line with OECD 221, under GLP compliance; all validity criteria were met. The RMS considers that this study is suitable for use in the risk assessment with a ErC_{50} of 2.35 mg a.s/L

B.9.2.8 Further testing on aquatic organisms

Acceptable risk to aquatic organisms from both napropamide-M and its metabolites has been shown based on the intended uses. Further studies to refine the risk assessment are therefore not required.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

Please refer to, Volume 3 – B9 (PPP), section B.9.5.1.

B.9.3.2. Effects on non-target arthropods other than bees

Please refer to Volume 3 – B9 (PPP), section B.9.5.2.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

Please refer to the DAR of napropamide-M, Volume 3 – B9 (PPP), section B.9.7.1.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

New studies have been carried out with napropamide-M with *Folsomia candida* and *Hypoaspis aculeifer* to fulfil the current data requirements for Regulation (EU) No. 283/2013. Study summaries are presented provided below.

Report

S. Vinall (2014) Napropamide-M tech- Laboratory determination of toxicity to the predatory mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate. UPL Europe Ltd, Unpublished report No.: UP-14-1

Guidelines

OECD guideline 226

GLP

Yes (certified)

Materials and Methods

Test material	Napropamide-M Technical
Description:	Orange-white crystals
Lot/Batch #:	UPH-08/DNE-263/TECH/20121226
Purity:	97.26 % w/w (total D- + L-isomer)

Solubility:	Insoluble in water.
Toxic Standard:	Dimethoate.
Negative control:	De-ionised water and a separate solvent control of acetone.

Test animals

Species:	*Hypoaspis aculeifer*
Age:	31 days (definitive test) after start of egg laying (approximately 7-14 days from becoming adult).
Source:	ECT Oekotoxikologie GmbH, Germany. (Test organisms from a culture maintained in the laboratory)

Experimental Dates: 26th of March 2014 – 6th of May 2014

Study Design

The predatory mite *Hypoaspis aculeifer* used in the study were cultured within the laboratory from a co-ordinate hatch to provide a uniform age of test organisms. Prior to the study the culture was maintained under similar conditions as those used for the definitive test.

The definitive test was carried out using one concentration of the test item (1000 mg a.s/kg dry weight soil) as a limit test. Napropamide-M has a low solubility in water and so it was necessary to prepare dispersions of the test item in acetone for application to the test substrate via a sand carrier. A 10.0 mL volume of the appropriate acetone solution was mixed with sand and the acetone allowed to evaporate away. Once the treated sand was dry it was then mixed with the other ingredients of the artificial soil to achieve 200 g dry weight of soil. Each aliquot of soil was then mixed with purified water to give a final moisture content of 50 % WHC.

The test substance was an artificial soil based on the requirements in the guideline containing 5% w/w Sphagnum peat with a pH of 6.0 ± 0.5. 20 g of treated soil was added to each of the test arenas (60 mL capacity glass jars (5.5 cm tall x 4.4 cm inner diameter) and lids with a hole for ventilation). Eight replicate arenas were used for each control and test item treatment and 5 replicates for the toxic reference treatment. Each replicate contained 10 female mites (to give a total of 50-80 mites per treatment). The mites were added to the treated soil within an hour of the soil being treated and as a food source Cheese mites (*Typrophagus putrescentiae*) and juvenile springtails (*Folsomia candida*) were added to the soil surface *ad libitum.* The arenas were weighed and water content amended to maintain 50 % WHC. For the definitive test, the ambient conditions were 19.8 - 20.4ºC, with a 16 h photoperiod of 450 - 625 lux.

The number of surviving mites and progeny in each test arena was assessed at 14 days. The soil from each arena was placed into individual Tullgren funnel apparatus consisting of a meshed container suspended over a funnel. Above the funnel a light bulb was fitted and for a period of 2 days with soil dried under the light forcing the *Hypoaspis aculeifer* to move downwards until they fell from the base of the funnel into the collecting vials containing 70 % alcohol beneath. Once removed the number of adult mites and juveniles were counted and the effects on mortality and reproduction in the treatment compared to the controls. The extraction efficiency was validated in a separate test, carried out by the test facility.

To determine the NOEC for mortality the 14-day data for the individual test-item treatments were compared to those for the acetone-control using Fisher's Exact Test ($\alpha = 0.05$). The mortality data was not suitable for Probit regression analysis (Finney, 1952) to calculate the median lethal concentration (LC_{50}) value. To determine the NOEC for reproduction a one-way ANOVA & Dunnett's t-test ($\alpha = 0.05$) was used. The reproduction data was not suitable for Probit regression analysis (Finney, 1952), to calculate the EC_{50} value.

Results and Discussion

According to Guideline 226 the validity criteria were met, the control mortality was 1% for the untreated control and 0% for the acetone control, the mean number of juveniles in control was at least

50 per replicate (300 for the negative control and 317 for the acetone control), the efficiency of the mite extraction method was > 95 % (99.2% for juvenile mites, 100% for adult females), the mean number of juveniles in the toxic reference treatment was at least 50 % lower than in the control (74% lower than the negative control and 75.5% lower than the acetone control).

In the control the coefficient of variation calculated for the number of juveniles was less than 30 at the end of the definitive test (8.8 in the negative control and 8.0 in the acetone control).

Biological results

The positive control study using Dimethoate 12 mg a.s./kg soil dry resulted in a 76% reduction in the mean number of progeny per replicate. The mean number of juveniles in the toxic reference treatment were at least 50% lower than in the control treatment (actual value in definitive test = 76%).

At 14 days the percentage mortality in the untreated control was 1 % and in the acetone-treated control was 0 %. The mortality in the treatment group of 1000 mg a.s/kg dry soil was 0 % and so the test item was not shown to have a toxic effect on *H. aculeifer*. The results of the reproduction assessment are summarised below.

Table B.9.4.2-1 Summary of reproductive effects and mortality on *H. aculeifer*

Treatment	Concentration (mg a.s/kg soil dry weight)[a]	Mean no. progeny per replicate[b]	% change relative to control[c]	Number of Live adult mites found at 14 DAT	% change relative to the negative control
Control I- untreated	-	300(± 26.3)	6	9.9(± 0.4)	1%
Control II- acetone	-	317(± 25.2)	-	10(± 0.0)	-
Napropamide-M Tech	1000	302(± 21.6)	5	10(± 0.0)	-
Toxic reference	-	78*(± 32.3)	76	3.4(± 2.2)	66%*

[a] Based on measured content of active ingredient (96.1 % w/w purity)
[b] The results for each treatment were individually compared to the acetone-control by one-way ANOVA and Dunnett's t-test) Values with * differed significantly from the acetone control
[c] A positive value indicates a decrease in reproduction, relative to the acetone-control

Conclusions

In the study on the effect of napropamide-M on the soil mite *Hypoaspis aculeifer*, the 14 day EC_{50} value for effects on fecundity was determined to be >1000 mg a.s/kg soil dry weight, the maximum concentration tested. The NOEC for assessments of both effects of mortality and fecundity was determined as being 1000 mg a.s/kg soil dry weight.

RMS Comments

It is noted that the EC_{50} for the toxic reference was 12 mg a.i./kg soil dry weight however the guideline suggests it should be in the range of 3-7 mg a.s./kg soil dry weight. This is considered to be a minor deviation that would not effect the outcome of the study. The study is considered to be suitable for use in the risk assessment.

This study was conducted in line with OECD 226, under GLP compliance; all validity criteria were met. The RMS considers that this study is suitable for use in the risk assessment with a NOEC of >1000 mg a.s/kg soil

Report

N. Geary (2015) Napropamide-M tech (HBW07) a laboratory determination of the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isotomidae). UPL Europe Ltd, Unpublished report No.: UP-15-1

Guidelines

OECD guideline 232

GLP

Yes (certified)

Materials and Methods

Test material	Napropamide-M Technical
Description:	Orange-white crystals
Lot/Batch #:	UPH-08/DNE-263/TECH/20121226
Purity:	96.1 % w/w (relating to D-isomer content),
Solubility:	Un-soluble in water.
Toxic Standard:	Betosip 114 (114 g/L phenmediphan)
Negative control:	De-ionised water and a separate solvent control of acetone.

Test animals	
Species:	*Folsomia candida*
Age:	11 days old for the definitive test.
Source:	In-house culture maintained at the Test Facility.
Experimental Dates:	14th of November 2014 – 11th February 2015.

Study Design

The test organisms *Folsomia candida* used in the study were cultured within the laboratory and from a co-ordinate hatch to provide a uniform age of test organisms. Prior to the study the culture was maintained in a controlled-environment room.

The definitive test was implemented using five concentrations of 1000, 555.6, 308.6, 171.5 and 95.3 mg a.s/kg soil dry weight. Napropamide-M has a low solubility in water and so it was necessary to prepare dispersions of the test item in acetone for application to the test substrate via a sand carrier. A 2 mL volume of the appropriate acetone solution was mixed with sand and the acetone allowed to evaporate away. Once the treated sand was dry it was then mixed with the other ingredients of the artificial soil to achieve 200 g dry weight of soil (containing 5% w/w Sphagnum peat). Each aliquot of soil was then mixed with purified water to give a final moisture content of 50 % WHC.

The test substrate was an artificial soil based on the requirements in the guideline with water content adjusted to 50% of the soil water holding capacity. The weight of each test arena was noted at the start of the test. At day 14, the arenas were reweighed, the change in the mean weight (through water loss from the soil) was calculated to be > 2% of the soil's original water content so additional purified water was added to restore the arenas' original weights. The weight of each arena was also measured at the end of the test. 30 g of treated soil was added to each of the test arenas. For the definitive test, 8 replicate arenas were used for each control 5 for the toxic reference treatment and 4 replicates per treatment concentrations. Each replicate contained 10 springtails to give a total of 40-80 individuals per treatment.

The test arenas were glass jars, approximately 125 mL capacity and 4.5 cm in diameter with a close fitting lid. Each replicate contained 10 springtails to give a total of 40-80 individuals per treatment. An additional jar was also prepared per treatment. For the definitive bioassay, the ambient conditions were 18.3 - 21.2ºC, with a 12h photoperiod of 510 - 790 lux. The pH of spare soil was measured (in the presence of 1 mol/L KCl) at the start and on day 14 of the test (5.82 – 6.24), using a GPH 014 meter and probe, this was maintained along with the other replicates throughout the test.

The weight of each test arena was noted at the start of the test. At day 14, the arenas were reweighed, the change in the mean weight (through water loss from the soil) was calculated to be > 2% of the soil's original water content so additional purified water was added to restore the arenas' original

weights. The weight of each arena was also measured at the end of the test. 30 g of treated soil was added to each of the test arenas.

The number of surviving mites and progeny in each test arena was assessed at 28 days. To make this assessment the test substrate from each arena was tipped in a tray. Water was then added to the substrate and stirred gently so the soil sank and the springtails floated to the surface and could be counted and removed. Black ink was also added to the water to help identify progeny present in the water, and individuals were counted using a binocular microscope. (In a separate test carried out by the test facility in October 2014 the efficiency of the extraction method was validated).

A toxic reference treatment of Betosip 114 (nominally 114 g/L phenmediphan) was applied at a concentration of 200 mg product/kg soil dry weight to assess the sensitivity of the test system.

For the mortality NOEC the data for the acetone-treated control were compared to the untreated control by Fisher's Exact Test ($\alpha = 0.05$). Since there was no significant difference, both control data were combined for comparison against the test-item treatments. The 28-day mortality data for the individual test-item treatments were compared to those for the combined controls using Fisher's Exact Test ($\alpha = 0.05$). The outcome of the bioassay was such that the median lethal concentration (LC_{50}) could not be determined by Probit regression analysis (Finney, 1952), but could be derived by extrapolation.

For the reproductive NOEC the numbers of F1 progeny in the individual treatment replicates were tested for normality of distribution (Shapiro-Wilk test, $\alpha = 0.05$) and treatments were also compared for homogeneity of variance (Levene's test, $\alpha = 0.05$) (Fowler & Cohen, 1990). Since the untreated control was not normally distributed both sets of control treatment data were compared by Mann-Whitney U-test ($\alpha = 0.05$). As there was no significant difference, both sets of control treatment data were combined for subsequent comparisons with the test-item treatments. Treatments were compared to the combined control data either by one-way analysis of variance and Dunnett's t-test (one-sided, $\alpha = 0.05$), or in the case of the toxic reference data, which failed the test for normality, by Mann-Whitney U-test (Fowler & Cohen, 1990).The outcome of the bioassay was such that the median effect concentration (EC_{50}) could not be determined by Probit regression analysis, but could be derived by extrapolation.

Results and Discussion

According to the Guideline 232 the validity criteria were met, the control mortality was 8.8% for the untreated control and 12.5% for the acetone control, the mean number of juveniles in the control was 621 in the untreated control and 598 in the acetone contro), the efficiency of the mite extraction method was 100% for the adult springtails and 98.3% for the juvenile springtails and the mean number of juveniles in the toxic reference treatment was 99 % lower than in the control.

In the control the coefficient of variation calculated for the number of juveniles was less than 30 at the end of the definitive test (8.8 in the negative control and 25.9 in the acetone control).

Biological results

The results of the experiment with the toxic reference treatment of Betosip 114 (nominally 114 g/L phenmediphan) which was applied at a concentration of 200 mg product/kg soil dry weight indicate that there was a 78% increase in mortality compared to the control and a 99.9% reduction in fecundity.

At 28 days the percentage mortality in the untreated control was 8.8 % and in the acetone-treated control was 12.5 %. The mortality reported from each of the treatment groups is presented in the following table. No significant effects on mortality were reported at any of the treatment concentrations tested.

Table B.9.4.2-2 Summary of mortality effects on *Folsomia candida*

Treatment[a]	Number of adult springtails found at 28 DAT	% Mortality[b]	Corrected Mortality[c]
Control- untreated	9.1 (± 1.1)	8.8	-

Control – acetone	8.8 (± 1.6)	12.5	-
1000 mg a.s/kg soil	8.0 (± 2.2)	20.0	10.5
555.6 mg a.s/kg soil	9.0 (± 0.0)	10	0.0
308.6 mg a.s/kg soil	9.8 (± 0.5)	2.5	0.0
171.5 mg a.s/kg soil	9.8 (± 0.5)	2.5	0.0
95.3 mg a.s/kg soil	8.25(± 0.5)	17.5	7.7
Toxic reference	2.2 (± 1.3)	78.0	75.4

[a] Based on measured content of active ingredient (96.1 % w/w purity)
[b] Mortality in the acetone-treated control and the untreated control did not differ significantly (Fisher's exact test $\alpha = 0.05$) so data combined. The remaining treatments were compared to the combined control data using Fisher's exact test and an * indicates a significant difference.
[c] Corrected for combined control treatment losses using Abbott's formula.

The effects on reproduction were assessed after 28 days. The results of the reproduction assessment are summarised below.

Table B.9.4.2- 3 Summary of reproductive effects on *Folsomia candida*

Treatment [a]	**Mean no progeny per replicate[b]**	**% change relative to the control[c]**
Control- untreated	621.6 (± 53.3)	-
Control – acetone	598.0 (± 154.8)	-
1000 mg a.s/kg soil	432.8* (± 83.5)	30.4
555.6 mg a.s/kg soil	460.0* (± 35.7)	29.0
308.6 mg a.s/kg soil	511.0 (± 58.6)	24.6
171.5 mg a.s/kg soil	525.3 (± 66.6)	16.2
95.3 mg a.s/kg soil	602.8 (± 57.9)	13.9
Toxic reference	0.4* (± 0.9)	99.9

[a] Based on measured content of active ingredient (96.1 % w/w purity)
[b] Fecundity data from the acetone and untreated controls were compared by Mann-Whitney U-Test ($\alpha = 0.05$), but did not differ significantly. Therefore fecundity data from individual test-item treatments were compared to the combined control data either by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$) or by Mann-Whitney U-test ($\alpha = 0.05$) Values marked with * indicate significant difference.
[c] A positive value indicates a decrease in reproduction, relative to the control.

Effects of > 50 % were not reported at any of the concentrations tested, but significant decrease compared to the control was reported at the two highest concentration tested of 555.6 and 1000 mg a.s/kg soil. The highest concentration tested without significant effect was 308.6 mg a.s/kg soil.

Conclusions

In the study on the effect of napropamide-M on the spring tail *Folsomia candida* the 28 day assessment on mortality and fecundity indicated that the LC_{50} value was >1000 mg a.s/kg soil and the NOEC to be 1000 mg a.s/kg .

RMS Comments

It is noted that the guideline recommends boric acid as a suitable toxic reference substance however Betosip 114 (114 g/L phenmediphan) has been used in this study. The applicant has stated that this is fit for purpose in the present study on the basis of its performance in past studies (i.e. historic data), the RMS considers this to be acceptable. At the proposed NOEC of 1000 mg a.s/kg soil 10% mortality was observed and a 30.4% reduction in fecundity therefore a more realistic NOEC of 95.3 mg a.s/kg soil is proposed for both mortality and fecundity. With this amendment the study is considered suitable for use in the risk assessment.

This study was conducted in line with OECD 232, under GLP compliance; all validity criteria were met. The RMS considers that this study is suitable for use in the risk assessment with a NOEC of >95.3 mg a.s/kg soil.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Please refer to the DAR of napropamide-M, Volume 3 – B9 (PPP), section B.9.9.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

Please refer to the DAR of napropamide-M, Volume 3 – B9 (PPP), section B.9.11.1.

B.9.6.2. Testing on non-target plants

Please refer to the DAR of napropamide-M, Volume 3 – B9 (PPP), section B.9.11.2.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

No other data are available on the effects of napropamide-M on other terrestrial organisms. As acceptable risk has been shown for all the standard test organisms, further testing on additional species is not considered necessary as the environmental risk from napropamide-M is considered to be acceptable with appropriate mitigation.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

The following study performed with napropamide is provided in support of the assessment. It was also submitted and evaluated during the EU review of napropamide. Therefore this study has not been re-evaluated by the RMS and the previously agreed Annex I endpoints have been used, where relevant, in the risk assessment.

REFERENCE: Annex IIA, 8.7 / 01

Report: J Hertl (2003) Toxicity of napropamide technical to activated sludge in a respiration inhibition test. United Phosphorus Ltd., Unpublished report No.: 18941171.

The effects of napropamide on activated sludge were investigated in one test with technical as. There was no inhibition at concentrations up to 1000 mg as/L (nominal). Therefore, the risk to sewage sludge treatment is considered to be acceptable.

Table 9.8-1: Summary of napropamide toxicity to biological methods for sewage sludge. Nominal concentration

Annex	Substance	Media	Process	EC_{50} (mg/L)
IIA, 8.7 / 01 Hertl, 2003	napropamide	Activated sludge	Respiration	> 1000

B.9.9. MONITORING DATA

No monitoring data have been submitted.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

No additional data submitted.

B.9.11. REFERENCES RELIED ON

Literature review

A literature review has been carried out for napropamide-M and any potential relevant metabolites. The review has been conducted in accordance with Article 8(5) of Regulation (EC) No. 1077/2009 and is based on the EFSA guidance document as published in EFSA Journal 2011; 9(2):2092.

The objective of the review was to determine if any scientific peer-reviewed open literature, published in the last 10 years before the submission date of the dossier (2005), was relevant for consideration during the risk assessment process described in this report. The conduct of the literature search method in relation to ecotoxicological studies has been evaluated; the conclusions of which are presented here.

Databases searched

The literature review involved a search of 12 databases (Anabstr - Analytical abstracts, Biosis, Caplus - chemical abstracts plus, Chemlist, Embase - The Excerpta Medica database, Scisearch, Toxcenter, Medline, Rtecs- Registry of Toxic Effects of Chemical Substances, Wiley online library, Science Direct, and Pubmed). Science Direct, PubMed and Wiley Online Library were found unable to process the volume and detail of the search terms used in the separate focused search strategies.

These databases cover a range of subject areas including those relevant to an ecotoxicological literature search.

Search Parameters

The search terms covered the names of the active substance, representative formulations, major metabolites and CAS numbers. These terms were combined with a range of detailed search terms specific to ecotoxicology.

The RMS evaluator notes that these detailed search terms do not consistently include Order level scientific terminology for invertebrates (for example along with terms such as ‘bug’, the search should include Diptera and Coleoptera). Amphibians have not been included in the search terms (reptiles have). Under Commission Regulation (EU) No. 283/2013 amphibians should be considered as part of the risk assessment based on the results of the literature review. Other than these issues, the additional search terms appear to cover a comprehensive range relating to those taxa considered as part of the ecotoxicological risk assessment.

Evaluation of studies for inclusion/exclusion based on relevance

An initial rapid assessment for relevance of the search results was conducted by review of the summary record titles and abstracts to exclude summary records which are obviously irrelevant. Following initial rapid assessment full text articles were assessed for relevance based on the criteria given in Table B.9.11-1.

It is noted that point 3 (relevant for EU regulatory risk assessment e.g. test species, endpoints identified, European conditions) is not appropriate as all species belonging to the groups considered as part of the risk assessment regardless of their distribution should be included. This is because toxicological effects on species not resident in Europe would still be valuable to consider during the risk assessment.

Table B.9.11-1: Criteria for relevance used to screen full text articles for use in ecotoxicology risk assessment

Data requirements (corresponding data points)	Criteria for relevance

Ecotoxicology literature (CA 8.1-8.8 and CP 10.1- 10.7)	1. Appropriate and clearly defined test material 2. Well characterised and relevant test system 3. Relevant for EU regulatory risk assessment e.g. test species, endpoints identified, European conditions. 4. Relevant to supported uses (or agricultural exposure in general) 5. Use of different formulation for field studies which would impact the exposure of NTOs to the active substance. 6. Availability of analytical data (where appropriate).

Search results

The result of this search method is summarised below in Table B.9.11-2.

Table B.9.11-2: Summary of ecotoxicological literature review search results

Data requirement(s) captured in the search	napropamide-M	Metabolites
Total number of summary records retrieved after limiting date spam, removal of patent documents and removal of duplicates:	450	1774
Number of *summary records* excluded from the search results after rapid assessment for relevance	450	1774
Total number of *full-text* documents assessed in detail	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0

Following a review of the justifications given for exclusion of the studies the RMS does not require any further clarification/full text copies of any additional studies.

Summary

The literature review appears to have been comprehensive and the methodology suitable with the above issues considered.

<u>References relied upon - active substance napropamide-M</u>

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.1.1.1/01	[redacted]	2013	Acute oral toxicity (LD_{50}) study of napropamide-	Y	Y	Data protection is claimed in accordance	UPL	None

			M technical in Japanese quail, *Coturnix coturnix japonica* Company report No. 516-3-08-6173 [redacted] GLP, Unpublished **Study submitted to meet data requirements**			with Article 59 of Regulation (EC) No 1107/2009		
CA 8.2.1/01	[redacted]	2011a	D-Napropamide: acute toxicity to rainbow trout (*Oncorhynchus mykiss*) in a 96-hour test Company report No. D03458 [redacted] GLP, Unpublished **Study submitted to meet data requirements**	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.2.2.1/01	[redacted]	2015a	Assessment of the effect of napropamide-M on fish, early life stage toxicity test Company report No. ENV-13-040 [redacted] GLP, Unpublished **Study submitted to meet data requirements**	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None

CA 8.2.2.3/01	[redacted]	1995	Napropamide: determination of the accumulation and elimination of [^{14}C] napropamide in bluegill sunfish (*Lepomis macrochirus*) Company report No. BL5352/B [redacted] GLP, Unpublished **Study submitted to meet data requirements**	Y	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.2.4.1/01	Liedtke, A.	2011c	D-Napropamide: acute toxicity to *Daphnia magna* in a 48-hour immobilization test Company report No. D03447 Harlan Laboratories Ltd., Switzerland GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.2.5.1/01	Kamle, M.K.	2014a	Reproductive output of *Daphnia magna* exposed to different concentrations of Napropamide-M technical over a period of 21 days	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None

			Company report No. 509-3-07-6174 Jai Research Foundation, India GLP, Unpublished **Study submitted to meet data requirements**					
CA 8.2.7/01	Ramsden, C.	2015b	Assessment of the effect of napropamide-M technical (HBW07) on *Lemna*, growth inhibition test + Amendment No 1. Company report No. ENV-13-046 AgroChemex Environmental Ltd, UK GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.2.7/02	Hermes, H.	2015	Napropamide-M (HBW07): Toxicity to the aquatic plant *Myriophyllum spicatum* in a semi static growth inhibition test with a prior rooting phase Company report No. 98011215 Ibacon GmbH, Germany GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None

CA 8.2.7/03	Juckeland, D.	2012a	Effects of napropamide metabolite Isomer I on *Lemna minor* in a growth inhibition test under semi-static test conditions Company report No. 11 10 48 017 W BioChem GmbH, Germany GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.2.7/04	Juckeland, D.	2012b	Effects of napropamide metabolite Isomer II on *Lemna minor* in a growth inhibition test under semi-static test conditions Company report No. 11 10 48 018 W BioChem GmbH, Germany GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.3.1.1.1/ 01	Rana, J.R.	2014a	Acute oral toxicity (LD_{50}) of D-devrinol 450 SC (HBW03) to the Honeybee, *Apis Mellifera* L Company report No. 523-3-08-6181 Jai Research	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None

			Foundation, India GLP, Unpublished **Study submitted to meet data requirements**					
CA 8.3.1.1.2/01	Rana, J.R.	2014b	Acute contact toxicity (LD_{50}) of D-devrinol 450 SC (HBW03) to the Honeybee, *Apis Mellifera* L Company report No. 523-3-08-6182 Jai Research Foundation, India GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.3.2.1/01	Gamblin, C.	2014	Acute dose-response toxicity of D-devrinol SC to the parasitic wasp *Aphidius rhopalosiphi* (De Stefani-Perez) (*Hymenoptera, Braxonidae, Aphidiinae*) Company report No. ENV-14-004 AgroChemex Environmental, Ltd, UK GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.3.2.2/01	Cockroft, R.	2014	Acute dose-response toxicity of D-	N	Y	Data protection is claimed in	UPL	None

			devrinol SC to the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Company report No. ENV-14-006 AgroChemex Environmental, Ltd, UK GLP, Unpublished **Study submitted to meet data requirements**			accordance with Article 59 of Regulation (EC) No 1107/2009		
CA 8.4.1/01	Rana, J.R.	2014c	Reproduction toxicity test of D-Devrinol 450 SC (HBW03) to earthworm, *Eisenia foetida foetida* Company report No. 522-3-08-6183 Jai Research Foundation, India GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.4.2.1/01	Vinall, S.	2014	Napropamide-M tech- Laboratory determination of toxicity to the predatory mite *Hypoaspis aculeifer* (Acari, Laelapidae) in an artificial soil substrate Company report No. UPL-14-1 Mambo-Tox, UK	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None

			GLP, Unpublished **Study submitted to meet data requirements**					
CA 8.4.2.1/02	Geary,N.	2015	Napropamide-M tech (HBW07) a laboratory test to determine the effects of fresh residues on the springtail *Folsomia candida* (Collembola, Isomidae) Company report No. UPL-15-1 Mambo-Tox, UK GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.5/01	Shrimali, A.	2013	Effect of d-Devrinol 450 SC (HBW03) on soil microorganisms – nitrogen transformation test Company report No. 608-3-15-6184 Jai Research Foundation, India GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.6.2/01	Dickinson, R.A.	2014a	d-Devrinol 450 SC Evaluation of the phytotoxicity to non-target	N	Y	Data protection is claimed in accordance with Article	UPL	None

			terrestrial plant – seedling emergence test Company report No. ACE-13-164 AgroChemex, Ltd, UK GLP, Unpublished **Study submitted to meet data requirements**			59 of Regulation (EC) No 1107/2009		
CA 8.6.2/02	Dickinson, R.A.	2014b	d-Devrinol 450 SC - Evaluation of the phytotoxicity to non-target terrestrial plant –Vegetative vigour test Company report No. ACE-13-165 AgroChemex, Ltd, UK GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None
CA 8.8/01	Hertl, J.	2003	Toxicity of Napropamide technical to activated sludge in a respiration inhibition test Company report No. 18941171 Ibacon, GmbH, Germany GLP, Unpublished **Study submitted to meet data requirements**	N	Y	Data protection is claimed in accordance with Article 59 of Regulation (EC) No 1107/2009	UPL	None