

European Commission



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

Mecoprop-P **Volume 3 – B.9 (AS)**

Rapporteur Member State : United Kingdom
Co-Rapporteur Member State :Ireland

Version History

When	What
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Table of contents

B.9. ECOTOXICOLOGY DATA	4
B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES	5
B.9.1.1. Effects on birds.....	5
B.9.1.2. Effects on terrestrial vertebrates other than birds	21
B.9.1.3. Active substance bioconcentration in prey of birds and mammals.....	21
B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)	21
B.9.1.5. Potential for endocrine disruption	21
B.9.2. EFFECT ON AQUATIC ORGANISMS	22
B.9.2.1. Acute toxicity to fish	22
B.9.2.2. Long-term and chronic toxicity to fish	28
B.9.2.3. Potential for endocrine disruption	34
B.9.2.4. Acute toxicity to aquatic invertebrates	34
B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates	35
B.9.2.6. Effects on algal growth.....	37
B.9.2.7. Effects on aquatic macrophytes	50
B.9.2.8. Further testing on aquatic organisms	54
B.9.3. EFFECTS ON ARTHROPODS	58
B.9.3.1. Effects on bees.....	58
B.9.3.2. Effects on non-target arthropods other than bees	77
B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA.....	85
B.9.4.1. Earthworm – sub-lethal effects	85
B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)	88
B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION	88
B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS	90
B.9.6.1. Summary of screening data	90
B.9.6.2. Testing on non-target plants	90
B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA).....	104
B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT.....	104
B.9.9. MONITORING DATA	108
B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER.....	108
B.9.11. REFERENCES RELIED ON.....	108

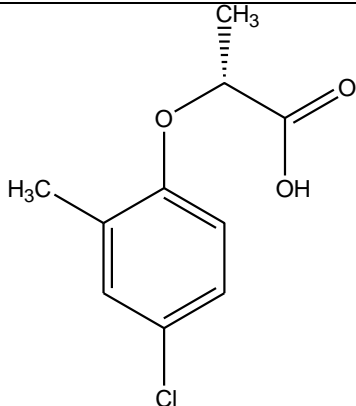
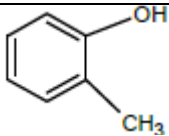
B.9. ECOTOXICOLOGY DATA

This document has been updated under the active substance renewal process under Regulation 844/2012. All new studies conducted to address the data requirements for active substance and potentially relevant metabolites are included in summary form below. Old studies from the original EU review of the active substance are also included in the original summary form. Any subsequent consideration of these studies by the RMS for renewal purposes is clearly noted in the supporting summary. Studies on both racemic mecoprop and mecoprop-P were considered in the 91/414/EC Review, which considered both mecoprop and mecoprop-P. It is considered that the studies on mecoprop-P are most relevant to this submission to support mecoprop-P only.

The active substance mecoprop-P ((R)-2-(4-chloro-o-tolyloxy)-propionic acid) is a phenoxy herbicide with a molecular mass of 214.65 and CAS No. 16484-77-8. Mecoprop-P is also referred to as MCP-P or mecoprop-P (acid) in the following sections.

For the environmental assessment a single metabolite of potential relevance is identified, with regards to the surface water compartment only: metabolite o-cresol, at up to 30.4 %. No other environmentally significant metabolites were found in soil, surface water, sediment or groundwater.

Table: B.9-01: Summary of the active substance and Environmental metabolites

Chemical name/ Trivial name	Structure	Environmental compartment
Mecoprop-P (R)- 2-(4-chloro-2-methylphenoxy) propanoic acid CAS 16484-77-8		Soil, Surface water, Sediment, Ground water, Air
O-cresol 2-methylphenol CAS 95-48-7		Surface water (max. 30.4%)

Analytical verification if studies submitted for renewal

For the purposes of active substance review under the new data requirements for Regulation 1107/2009 (as outlined in Regulation 283/2013 and Regulation 284/2013) it is a requirement to present the details and supporting validation data for pre-registration methods. Such methods are evaluated for the studies submitted in support of the renewal of mecoprop-P in Volume 3 section B.5.1.2.6 of the active substance (CA) and product (CP) Assessment Reports.

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES**B.9.1.1. Effects on birds****B.9.1.1.1. Acute oral toxicity to Birds**

Ref.: IIA. 8.1.1. Grimes, 1986 a: MCPP: An acute oral toxicity study with the Mallard duck.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute oral toxicity of MCPP, >92% pure, to birds was studied on Mallard duck (*Anas platyrhynchos*) according to US-EPA guideline FIFRA Subdivision E, 71-1. Six groups of 10 birds, 5 males and 5 females, were used in each treatment group. The birds were 30 weeks of age when they were given a single oral dosage of nominal 0, 292, 486, 810, 1350 and 2250 mg/kg bodyweight (bw) dispersed in corn oil. The observation period was 14 days. The birds weighed from 856 g to 1259 g.

Results

There was 10% mortality at 2250 mg/kg bw and none in any other dosage group. The mortality pattern was not conducive enough to calculate the LD₅₀ value which was estimated based on visual inspection. One bird was regurgitating at 292 mg/kg bw but none at 486 mg/kg bw. At 810 mg/kg bw, 8 birds were regurgitating after dosing and lethargy was noted two hours after dosing. All birds except one regurgitated at 1350 and 2250 mg/kg bw and overt signs of typical intoxication including lethargy, ruffled appearance, wing drop, loss of coordination, convulsions and coma were observed temporarily. The acute oral LD₅₀ was determined to be greater than 486 mg/kg bw, the level at which no regurgitation was observed. NOEC was less than 292 mg/kg bw.

RMS Comments

LD₅₀ can not be calculated as the dosages are unknown because of the regurgitations. However, MCPP appeared not to be acute toxic to the Mallard duck and the report conclusion that LD₅₀ is above 486 mg/kg was acceptable.

RMS comments (renewal):

Due to the observed regurgitations and failure to define a robust LD₅₀ value it is proposed by the RMS to make preferential use of other submitted/available acute avian toxicity data. Studies on racemic material (with which the above study was conducted) were previously evaluated but are now disregarded as there is sufficient data on mecoprop-P for this evaluation.

Ref.: IIA. 8.1.1. Munk, 1986 a: Avian Single-dose oral LD₅₀ of MCPP (Mecoprop), TPH batch to the Bobwhite quail (*Colinus virginianus*).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute oral toxicity to birds of technical MCPP acid (92.7% pure) was studied on Bobwhite quail (*Colinus virginianus*) according to US-EPA FIFRA 158.145, 71-1. Groups of ten birds were used per dosage; 5 males and 5 females. The birds were dosed by gavage of single doses of 0, 125, 250, 500, 1000 and 2000 mg/kg bw followed by a 14 day observation period.

Result

No mortality occurred in the 0 to 500 mg/kg bw dose groups. At 1000 and 2000 mg/kg bw, 100% mortality was observed after 4 and 2 days, respectively. LD₅₀ was determined at 500-1000 mg/kg bw (> 500 mg/kg bw and < 1000 mg/kg bw).

In the 500 mg/kg bw group and higher severe toxic signs such as apathy, side- and prone position, ruffled feathers, ataxia and diarrhoea were observed. The mean total feed consumption/bird/day during the 14 day observation period was in the same order of magnitude in all test groups. The initial feed consumption/bird/day was reduced dose dependent in the 250 and 500 mg/kg bw groups. Nearly no feed was taken up by the birds in the two highest dosage groups. General congestive hyperemia was observed in the birds that died (1000 and 2000 mg/kg bw). NOEL was determined as 125 mg/kg bw.

Table B.9.1.1-01: Results on acute toxicity to Bobwhite quail

Effect	Test doses, mg/kg bw					
	0	125	250	500	1000	2000
Mortality %	0%	0%	0%	0%	100%	100%
Mean consumption male g/bird/day (1) female	15.4 14.8	16.5 15.4	14.1 11.9	9.5 5.9	5 6	1 3
Mean consumption male g/bird/day (2) female	15.3 16.7	17.0 17.1	16.5 16.4	15.1 14.5		

(1): male and female, mean feed consumption/day during day 0-3

(2): mean of means during day 0-3, 4-7 and 8-14

RMS Comments

The study is acceptable.

RMS comments (renewal):

Study not revisited. The LD₅₀ from the study is confirmed as >500 mg a.s./kg bw. Studies on racemic material (with which the above study was conducted) were previously evaluated but are now disregarded as there is sufficient data on mecoprop-P for this evaluation.

Ref.: IIA. 8.1.1. Munk, 1987: Avian Single-dose oral LD₅₀ of MCP (D-form) to the Bobwhite quail (*Colinus virginianus*).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute oral toxicity to birds of MCP (D-form = MCP-P, >92.3% pure) was studied on Bobwhite quail (*Colinus virginianus*) according to US-EPA 71-1. Groups of ten birds, about 7 months old, were used per dosage; 5 males and 5 females. The birds were dosed by gavage of single doses of 0, 62.5, 125, 250, 500 and 1000 mg/kg bw followed by a 14 day observation period.

Results

No mortality occurred in 0 to 250 mg/kg bw. At 500 and 1000 mg/kg bw, 40% and 100% mortality were observed after 3 and 2 days, respectively. LD₅₀ was determined as 500 mg/kg bw.

In 250 mg/kg bw group one male showed apathy on the day of administration and on day one males and females had soft faeces. In the higher dose groups, severe toxic signs such as apathy, prone and side position, ruffled feathers, ataxia and diarrhoea were observed. The mean total feed consumption /bird/day during the 14 day observation period was in the same order of magnitude in all test groups. The initial feed consumption/bird/day was reduced - dose dependent - in the 250 to 1000 mg/kg bw groups. NOEL was determined as 125 mg/kg bw.

Table B.9.1.1-02: Cumulative mortality and feed consumption

Effect	Effect at test dose mg/kg bw					
	0	62.5	125	250	500	1000
Mortality %	0%	0%	0%	0%	40%	100%
Mean consumption male	16.1	13.8	13.9	11.9	7.3	-
g/bird/day (1) female	16.5	13.9	12.3	13.1	4.1	-
Mean consumption male	14.3	13.4	13.3	14.1	13.2	-
g/bird/day (2) female	13.9	12.3	13.0	14.4	11.7	-
Body weight male	197	196	193	194	192	-
day 14 female	180	186	187	186	185	-

(1): male and female, mean feed consumption/day during day 0-3. (2): mean of means during day 0-14

RMS Comments

The study is acceptable. The LD₅₀ was determined as 500 mg MCPP-P/kg bw for Bobwhite quail.

RMS comments (renewal):

The RMS has revisited the study report in question for the purpose of active substance renewal. The validity criteria (according to modern EPA guideline OPPTS 850.2100) were met, as control mortality did not exceed 10% (0% after 14 days). Also key attributes of the study methodology were achieved: At least 10 birds per treatment group were used (10, both male and female); there were at least 5 tested dose groups (5); animals were acclimated to test conditions for at least 14 days and the study duration was at least 14 days.

The study is considered to be valid and suitable to derive an endpoint for risk assessment purposes.

The agreed endpoint is a 14-day LD₅₀ = 500 mg a.s./kg bw as mecoprop-P

Ref.: IIA. 8.1.1; IIIA. 10.1.1. Pedersen & Helsten, 1992 a: R(+)-2-(2-methyl-4-chlorophenoxy)-propionic acid dimethylamine salt (MCPP-P DMAS): 14-day acute oral LD₅₀ study in Bobwhite quail.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute oral toxicity to birds of mecoprop-P dimethylamine salt (MCPP-P DMA) with the purity 65.62% as DMA salt and 54.23% as acid was studied on Bobwhite quail (*Colinus virginianus*) according to US-EPA Subdivision E, 71-1. Groups of ten 22 weeks old birds were used per dosage; 5 males and 5 females. The birds were dosed by gavage of single doses of 0, 191, 318, 529, 882 and 1470 mg MCPP-P DMA/kg body weight followed by a 14 day observation period.

Results

Chalky diarrhoea was noted in all groups, however, the animals recovered after day 2 at 191 and 318 mg/kg dose level. Lethargy and hyper-salivation were observed at the highest dose level. Mortality occurred from 529 mg/kg and all birds died at the two highest dose levels. LD₅₀ was determined as 602 mg MCPP-P DMA/kg bw. with confidence limits 489-740 mg/kg bw. When calculated as free acid LD₅₀ was 497 mg MCPP-P/kg bw.

The body weight was reduced at 529 mg/kg bw concurrent with reduced feed consumption. Gross pathological findings that could be related to the test substance were intestinal hemorrhages and fluid filled crop in dead animals. NOEL was 318 mg MCPP-P DMA/kg bw based on body weight, but including the observations of transient diarrhoea it would be <191 mg MCPP-P DMA/kg bw.

Table B.9.1.1-03: Results on acute toxicity to Bobwhite quail

Effect	Test doses, mg/kg bw					
	0	191	318	529	882	1470
Mortality %	0%	0%	0%	30%	100%	100%

Body weight (g) day 1	189	193	192	193	196	193
day 3	195	200	193	176	-	-
day 7	201	210	201	189	-	-
day 14	213	216	212	202	-	-
Mean consumption (1)	20	18	18	11	-	-
g/bird/day (2)	20	24	21	16	-	-

∴ No survivors. (1): Estimated feed consumption per bird per day (g) during day 1-3. (2): Estimated mean consumption, mean of 4-7 and 7-14.

RMS Comments

The study is acceptable. The documentation was placed under preparation toxicity (IIIA. 10.1.1) without comments on which preparation was used. The text refers to a batch 9158/7 probe 31. The results are corrected for the active ingredient content of the test material.

RMS comments (renewal):

The RMS has revisited the study report in question for the purpose of active substance renewal. The validity criteria (according to modern EPA guideline OPPTS 850.2100) were met, as control mortality did not exceed 10% (0% after 14 days). Also key attributes of the study methodology were achieved: At least 10 birds per treatment group were used (10, both male and female); there were at least 5 tested dose groups (5); animals were acclimated to test conditions for at least 14 days (15 days) and the study duration was at least 14 days.

The study is considered to be valid and suitable to derive an endpoint for risk assessment purposes.

The agreed endpoint is a 14-day LD₅₀ = 602 mg a.s./kg bw as MCP-P DMA, equivalent to 497 mg a.s./kg bw as MCP-P acid.

Report:	CA 8.1.1.1/01 [REDACTED] (1995)
Title	Mecoprop-P acute oral toxicity (LD50) to bobwhite quail Report No. RNP 445/950668
Guidelines:	US EPA, Subdivision E, Series 71, §71-1
GLP:	Yes
Deviations	None

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

- Test materials:** Mecoprop-P technical
Description: Light brown powder
Lot/Batch #: DA 928
Purity: 89.7 % w/w
CAS #: 16484-77-8
Stability of test compound: Stable
- Vehicle and/or positive control:** Corn oil

3. Test animals

Species:	Bobwhite quail (<i>Colinus virginianus</i>)
Age:	11 months
Weight at dosing:	160 - 216 g
Source:	[REDACTED]
Acclimatisation period:	15 days
Diet:	Standard layer diet from Parker Brothers Ltd, <i>ad libitum</i> with the exception of a 22 h starvation period prior to dosing
Water:	Filtered tap water, <i>ad libitum</i>
Housing:	Housed by sex in groups of 2 or 3 in a plastic coated steel wire mesh cage of dimensions 31 x 39 x 24 cm.

4. Environmental conditions

Temperature:	19 – 21°C
Humidity:	72% mean relative humidity
Air changes:	Not reported, ventilation reported as provided.
Photoperiod:	10 hours of artificial light, 14 hours dark.

B. STUDY DESIGN

1. In life dates:

24 August – 22 September 1994

2. Animal assignment and treatment

The birds were allocated to treatment groups on the basis of bodyweight, with the aim of all treatment groups having similar mean bodyweights and bodyweight distributions. Groups were assigned to treatment using a random allocation procedure. 5 pairs (i.e. 5 male and 5 female birds) were dosed per definitive test group.

3. Dose selection rationale

Dose levels were selected on the basis of range finding studies, in which groups of 2 birds were dosed at 250, 500 or 1000 mg/kg bw. Both birds in the 1000 mg/kg b.w range finding group died. There were no deaths in the 250 and 500 mg/kg bw treatment groups.

Dose levels were selected at 0 (control), 260, 364, 510, 714 or 1000 mg Mecoprop-P/kg bw for the main test.

4. Preparation of dosing mixtures

Birds were given a single dose of the test material in corn oil by oral intubation. Care was taken to ensure that birds had ingested the entire dose before being returned to their cage. Control animals were subject to the same procedure, but received vehicle only. All birds received the same volume per unit of bodyweight.

5. Statistics

Changes in body weight were analysed using the Williams, 1971 & 1972 Analysis of Variance test.

C. METHODS

1. Observations

Birds were observed daily during the study and at frequent intervals during the post-treatment period. Mortalities, bird health and clinical signs were recorded at each observation.

2. Body weight

The bodyweight of each bird was recorded on days -15, -7, 0 (immediately prior to dosing), 7 and 14.

3. Food consumption

Group mean food consumption was recorded over days -15, to -8, -7 to -1, 1 to 7 and 8 to 14.

4. Macroscopic examination

Any bird which died during the study was examined post mortem. At termination of the study post mortem examination was carried out on all control birds and all ten birds from the highest surviving dose group.

II. RESULTS AND DISCUSSION

1. Clinical signs of toxicity

There were no clinical signs in the control and 260 mg/kg bw groups. The birds treated at 364 mg/kg bw all showed subdued behaviour, which has fully resolved before the end of Day 1.

Subdued behaviour, unsteadiness and ruffled feathers were observed in all birds in the 510, 714 and 1000 mg/kg bw treatment groups. All surviving birds recovered by Day 4 and Day 5 in the 510 and 714 treatment groups respectively.

2. Mortality, Bodyweight gain, food consumption

Table B.9.1.1-04: Effects of mecoprop-P on mortality, bodyweight and food consumption of *C.virginianus*

Administered dose (mg a.s./kg bw)	14-day mortality (%)	14-day Bodyweight gain (g)	Bodyweight gain (%)	D1-7 food consumption (g/bird/day)	D8-14 food consumption (g/bird/day)
Control (0)	0	Male = 13 Female = 10	Male = 7.2 Female = 5.0	Male = 20 Female = 26	Male = 21 Female = 28
260	0	Male = 9 Female = 14	Male = 5.0 Female = 7.1	Male = 18 Female = 22	Male = 21 Female = 26
364	0	Male = 11 Female = 7	Male = 6.1 Female = 3.5	Male = 16 Female = 18	Male = 21 Female = 25

Administered dose (mg a.s./kg bw)	14-day mortality (%)	14-day Bodyweight gain (g)	Bodyweight gain (%)	D1-7 food consumption (g/bird/day)	D8-14 food consumption (g/bird/day)
510	10	Male = 9 Female = 8	Male = 5.0 Female = 4.0	Male = 15 Female = 15	Male = 19 Female = 22
714	70	Male = -1 Female = 1	Male = -0.5 Female = 5.5	Male = 12 Female = 13	Male = 22 Female = 24
1000	100	Male = n/a Female = n/a	Male = n/a Female = n/a	Male = 9 Female = 24	n/a (all birds dead)

3. Post-mortem macroscopic effects

No abnormalities were noted in any of the birds that were examined.

4. Validity Criteria

Under the modern equivalent EPA guideline (OCSPP 850.2100) the Avian Acute Oral Toxicity Test can be considered valid if the following criteria are met:

- Birds are randomly assigned to each treatment and control group: The report confirms that birds were randomly assigned but considering bodyweights so that all treatment groups had similar mean bodyweights and bodyweight distributions
- Control group mortality does not exceed 10%: 0%
- There are at least 10 individuals per group in the definitive test: 5 males + 5 females per group
- Administration of the test item is by capsule or oral gavage: Oral gavage used
- For the definitive test there are at least 5 tested doses and a control group: vehicle control plus 5 dose groups in the definitive test

III. CONCLUSIONS

Based on the results of this study, the acute oral median lethal dose (LD_{50}) was found to be 648 mg/kg bw.

RMS comments:

Despite its age the study was well reported and conducted in close adherence with the modern (2012) version of the corresponding EPA guideline. All validity criteria were met and study methodology was in line with that recommended for the tested species. The study is considered valid and acceptable for risk assessment purposes.

The agreed study endpoint is a 14-day LD_{50} = 648 mg a.s./kg bw

B.9.1.1.2. Short-term dietary toxicity to birds

Ref.: IIA. 8.1.2. Grimes, 1986 b: MCP: A dietary LC₅₀ study with the Mallard.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The dietary toxicity of MCP, > 92% pure, on birds was studied on Mallard ducks (*Anas platyrhynchos*) according to US-EPA FIFRA subdivision E 71-2 and ASTM E 857-81. The birds were 9 days old when they in groups of 10 birds were exposed to a nominal concentration of 562, 1000, 1780, 3160 and 5620 ppm mecoprop in the food for 5 days. The birds were observed for an additional 3 days.

Results

The LC₅₀ was determined to be greater than 5620 ppm which was the highest concentration tested because there was no mortality or overt signs of toxicity during the test period. A reduction in body weight gain was observed at 3160 ppm and 5620 ppm concentrations during the exposure. The NOEC was determined to be 1780 ppm based on the effects on body weight gain.

Table B.9.1.1-05: Body weight and estimated feed consumption

Effect	Effect after exposure to MCP in food (ppm):					
	0	562	1000	1780	3160	5620
Body weight (g)						
Day 0	144	144	138	132	132	137
weight gain	116	109	110	109	89	30
Day 5	260	253	248	241	221	167
weight gain	93	103	109	98	102	117
Day 8	353	356	357	339	323	284
total weight gain	209	212	219	207	191	147
Consumption, g/bird/day						
Days 0-5	50	47	48	42	46	29
Days 6-8	69	75	74	72	76	74

RMS Comments

The exposed birds regain their weights when they are no longer exposed. The study is acceptable.

RMS comments (renewal):

The study report has been revisited by the RMS for the purposes of active substance renewal. There was less than 10% control mortality, the exposure length was at least 5 days and total duration was at least 8 days.

The LC₅₀ is calculated as > 5620 mg/kg feed. Based on mean bird body weight during the dose period (mean = 147 g), mean food consumption during dosing (27 g/bird/day) and stated test item purity (92%) the LD₅₀ is calculated to be > 1020 mg MCP/kg bw/day.

However, it is noted that no analytical verification of the test item concentrations in feed was made. As such the study endpoint is not considered to be reliable and so the study should not be used in the risk assessment. The measurement of achieved test item concentrations is a requirement of the modern EPA guideline 850.2200 (1996 version onwards). The study is considered to be superseded by Munk, R. (1996) which was submitted for the purposes of renewal. Studies on racemic material (with which the above study was conducted) were

previously evaluated but are now disregarded as there is sufficient data on mecoprop-P for this evaluation.

Ref.: IIA. 8.1.2. Munk, 1986 b: Avian dietary LC₅₀ test of MCP (Mecoprop; TPH batch to the Bobwhite (Colinus virginianus)).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The dietary toxicity of MCP, TPH-batch 92.7% pure, was studied on Bobwhite quails (*Colinus virginianus*) according to US-EPA FIFRA subdivision E 71-2 (1982). The birds were 14 days old when they in groups of 10 birds were exposed to a nominal concentration of 0, 313, 625, 1250, 2500 and 5000 ppm mecoprop in the diet for 5 days. The birds were observed for an additional 3 days.

Results

The LC₅₀ was determined to be about 5000 ppm. At 2500 ppm no mortality occurred. At the highest concentration tested 50% of the exposed birds died. No signs of toxicity were observed except apathy in the surviving birds at 5000 ppm. A reduction in feed consumption was observed at 5000 ppm during the exposure period. The NOEC was determined to be 2500 ppm.

Measurements of the concentration varied 98 to 107% from the nominal concentration.

Table B.9.1.1-06: Body weight and estimated feed consumption

Effect	Effect after exposure to MCP (ppm):					
	0	313	625	1250	2500	5000
Mortality, %	0%	0%	0%	10%	0%	50%
Body weight (g)						
Day 0	27.8	26.5	27.3	27.2	27.1	26.4
weight gain	8.6	7.4	8.0	6.7	6.0	1.3
Day 5	36.4	33.9	35.3	33.9	33.1	27.7
weight gain	7.7	6.0	7.2	8.4	6.5	6.1
Day 8	44.1	39.9	42.5	42.3	39.6	33.8
total weight gain	16.3	13.4	15.2	15.1	12.5	7.4
Consumption, g/bird/day						
Days 0-5	4.6	4.4	4.6	4.6	4.6	3.5
Days 6-8	6.1	5.2	6.7	5.6	6.5	6.1

RMS Comments

The study was acceptable. No statistically significant reduction in body weight occurred although a slight reduction in body weight gain occurred in the highest dose group.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. It is noted by the RMS that dietary toxicity is not standardly considered under the current EFSA (2009) avian risk assessment. As such the study has not been revisited for renewal purposes. Studies on racemic material (with which the above study was conducted) were previously evaluated but are now disregarded as there is sufficient data on mecoprop-P for this evaluation.

Ref.: IIA. 8.1.2. Pedersen & Helsten, 1992 b: R(+)-2-(2-Methyl-4-chlorophenoxy)-propionic acid dimethylamine salt (MCP-P DMAS): 8-Day acute dietary LC₅₀ study in Bobwhite Quail.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The dietary toxicity of MCP-P DMAS (dimethylamine salt, 65.6% as optically active salt, equivalent to 54.23% as optically active acid), was studied on Bobwhite quails (*Colinus virginianus*) according to US-EPA FIFRA subdivision E 71-2 (1982).

The birds were 11 days old when they in groups of 10 birds were exposed to MCP-P DMAS in a nominal concentration of 0, 350, 700, 1400, 2800 and 5600 ppm a.s. in the diet for 5 days. The birds were observed for an additional 3 days. 5 groups of 10 birds were exposure for the control group

Results

The LC₅₀ was determined to be > 5600 ppm. No mortality or signs of toxicity were recorded during the study. A reduction in body weight and feed consumption were observed at 2800 ppm and 5600 ppm during the exposure period. The NOEC was determined to be 1400 ppm a.s. Calculated as acid equivalents the corresponding values would be: LC₅₀ > 4630 ppm and NOEC = 1160 ppm.

Measurements of the concentration varied 84.8 to 146.6% from the nominal concentration.

Table B.9.1.1-07: Body weight and estimated feed consumption

	Effect after exposure to MCP in food at (ppm):					
	0	350	700	1400	2800	5600
Mortality, %	0%	0%	0%	0%	0%	0%
Body weight (g)						
Day 0	26	26	26	25	27	25
weight gain	15	15	17	16	11	2
Day 5	41	41	43	41	38	27
weight gain	11	10	10	10	11	12
Day 8	52	51	53	51	49	39
total weight gain	26	25	27	26	22	14
Consumption, g/bird/day						
Days 1-5	6	6	6	6	5	4
Days 6-8	8	8	8	8	8	7

RMS Comments

The study was acceptable. The documentation was placed under active substance toxicity (IIA. 8.1.2) without comments on whether technical or pure substance or a preparation was used. The text refers to a batch 9158/7 probe 31 as in Pedersen & Helsten (IIIA. 10.1.1). The results are corrected for the active ingredient content of the test material.

RMS comments (renewal):

The RMS has revisited the study report for the purposes of active substance renewal. In accordance with the EPA guideline OPPTS 850.2200 (1996 version) the study can be considered valid. Control mortality did not exceed 10%, dose concentrations in the feed were all at least 80% of nominal and other methodology was adhered to. The 8-day LC₅₀ is confirmed as > 5600 mg a.s./kg feed (as dimethylamine salt). The LD₅₀ is calculated by the RMS to be >861.5 mg a.s./kg bw/day as MCP-P DMA salt, based on nominal food

concentrations, mean consumed feed per day, and mean chick bodyweight in the highest test group. Expressed as MCPP-P the endpoint is > 712.2 mg a.s./kg bw/day.

Report:	CA 8.1.1.2/01 [REDACTED] (1996)
Title	MCPP-P-DMA Salt – Avian Dietary LC ₅₀ test in chicks of the mallard duck (<i>Anas platyrhynchos</i> L.) Report No. [REDACTED]
Guidelines:	US EPA, Subdivision E, Series 71, §71-2
GLP:	Yes
Deviations	Minor deviations to modern guidelines

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

1. **Test materials:** Mecoprop-P DMA salt

Description: Brownish liquid

Lot/Batch #: 91-1 9158/7

Purity: 741.2 g Mecoprop-P DMA/L, equivalent to 617.6 g Mecoprop-P (acid)/L

CAS #: 66423-09-4

Stability of test compound: Stable over test duration (confirmed via HPLC analysis of feed samples with similar properties)

Density: 1.13 mg/mL
2. **Vehicle and/or positive control:** Diet
3. **Test animals**

Species: Mallard duck (*Anas platyrhynchos* L.)

Age: 7 days

Weight at dosing: 42.4 – 100.9 g

Source: [REDACTED]

Acclimatisation period: 6 days in lab, 2 to study cages and conditions

Diet: Sniff diet (17.5% crude protein), *ad libitum*

Water: Filtered tap water, *ad libitum*

Housing: Housed in groups of 10 in wire mesh cages 130 x 65 x 130 cm, with ceramic heater above each cage.
4. **Environmental conditions**

Temperature: 20 ±2°C in room, 37-38°C directly under heater

Humidity:	50 – 60%
Air changes:	Not reported
Photoperiod:	16 hours of artificial light, 8 hours dark at 165 – 240 lux

B. STUDY DESIGN

1. In life dates:

04 January – 12 January 1996

2. Animal assignment and treatment

The birds were randomly allocated to treatment groups on the basis of bodyweight. A single cage of 10 chicks was used per control and dose concentration group. Chicks were fed dosed diet *ad lib* over a 5 day period, followed by a non-dosed 3 day observation period.

3. Dose selection rationale

5000 ppm diet was selected as the standard highest dose. Dose concentrations were then spaced by a factor of 2, resulting in test concentrations of 0, 313, 625, 1250, 2500 and 5000 ppm.

4. Preparation of dosing mixtures

Each concentration was mixed separately by preparing a premix. The premix was then mixed with meal to form basal diet in a laboratory mixer. The final mixtures were stored in containers at room temperature. Stability and homogeneity was confirmed. Analysis of feed samples confirmed the achieved concentrations of the test item.

5. Statistics

Statistical evaluation of bodyweight was performed by one-way analysis (ANOVA) followed by Dunnett's test.

C. METHODS

1. Observations

Birds were observed daily during the study. Mortalities, bird health and clinical signs were recorded at each observation.

2. Body weight

The bodyweight of each bird was recorded on days 0, 5 and 8.

3. Food consumption

Mean bird food consumption was recorded over days 1 to 5 and 6 to 8.

4. Macroscopic examination

Any bird which died during the study was examined post mortem. At termination of the study post mortem examination was carried out on all birds.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION OF CONCENTRATIONS

Analysis of the prepared dosed feed was analysed for concentrations of the test item (as DMA salt). The following concentrations were achieved:

- Control group: analysed content = not detected
- 313 mg a.s./kg = 303 mg test item/kg → 198.7 mg a.s./kg diet
- 625 mg a.s./kg = 1165 mg test item/kg → 764.2 mg a.s./kg diet
- 1250 mg a.s./kg = 1743 mg test item/kg → 1143 mg a.s./kg diet
- 2500 mg a.s./kg = 3866 mg test item/kg → 2536 mg a.s./kg diet
- 5000 mg a.s./kg = 7205 mg test item/kg → 4726 mg a.s./kg diet

B. OBSERVATIONS

1. Clinical signs of toxicity

Apathy and ataxia was observed in one bird in the 5000 ppm group on Day 1.

2. Mortality, Bodyweight gain, food consumption

Table B.9.1.1-08: Effects of Mecoprop-P-DMA salt on mortality, bodyweight and food consumption of *A. platyrhynchos*

Dose (mg a.s./kg feed)		14-day Mortality (%)	8-day Bodyweight gain (g)	Bodyweight gain (%)	D1-5 food consumption (g/bird/day)	D6-8 food consumption (g/bird/day)
nominal	measured					
Control (0)	0	0	121.2	170	42	65
313	198.7	0	131.0	184	42	72
625	764.2	0	135.6	189	42	70
1250	1143	0	97.9	152	31	49
2500	2536	0	91.2	135	35	59
5000	4726	10	68.0	99	23	52

3. Macroscopic post-mortem observations

No abnormalities were noted in any of the birds that were examined.

4. Validity criteria

Under the modern equivalent EPA guideline (OCSPP 850.2200) the Avian Dietary Oral Toxicity Test can be considered valid if the following criteria are met:

- Birds are randomly assigned to each treatment and control group: The report confirms that birds were randomly assigned but considering bodyweights so that all treatment groups had similar mean bodyweights and bodyweight distributions
- Control group mortality does not exceed 10%: 0%
- Concentrations of the test item are satisfactorily maintained in the diet over the 5 day dosing period: Stability of the test item in diet was demonstrated, endpoints were based on achieved measured concentrations in diet.
- Continuous dosing must take place for 5 consecutive days. *Ad libitum* dosed feed was provided for a 5 day period.
- There are at least 10 individuals per group in the definitive test: 10 chicks per control/concentration tested
- Administration of the test item is by diet: administered as mix in feed
- For the definitive test there are at least 5 tested doses and a control group: vehicle control plus 5 dose groups in the definitive test

III. CONCLUSIONS

Based on the results of this study, the acute oral median lethal concentration (LC₅₀) was >4726 mg Mecoprop-P DMA/kg diet (ppm). The NOEC was 764.2 mg Mecoprop-P DMA/kg diet (ppm), based on measured concentrations of the test item.

RMS comments:

Despite its age the study was well reported and conducted in close adherence with the modern (2012) version of the corresponding EPA guideline. All validity criteria were met but due to the age of the study there were some variations from recommended parameters for the tested species. EPA 850:2200 recommends use of 5 day old chicks, whereas this study used 7-day old chicks, the protein content in the diet was also below the recommended 25% in the modern guideline. Also the light cycle utilised was 16:8, as opposed to the recommended 14:10. These are considered to be minor deviations that do not impact the validity of the study endpoint, as control group performance was satisfactory. Overall the study is considered valid and acceptable.

The agreed study endpoint is an 8-day LC₅₀ > 4726 mg mecoprop-P/kg diet (ppm), based on measured concentrations of the test item. Based on the average chick weight over the 8-day period exposed this tested concentration, alongside the average food intake per day over the 5-day exposure period, this is calculated by the RMS to correspond to an **LD₅₀ > 1051.7 mg a.s./kg body weight/day as DMA salt, equivalent to > 876.4 mg a.s./kg bw/day as MCPP-P (acid).**

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

Reference

██████████ (1999): Technical mecoprop-P DMA. Effects on reproduction in Japanese quail after dietary administration. ██████████ Report No. RNP 594/985229. MCPP-P Task Force, BASF Doc ID 1999/10910.

Previous evaluation:	Included in Addendum II to DAR (July 2002) for original a.s. approval
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Methods

The effects of dietary administration of technical mecoprop-P DMA on reproduction in Japanese quail was studied according to OECD draft test guideline on bird reproduction, 1996.

The test species, Japanese quail *Coturnix japonica*, were approx. 12 weeks old at the start of the study. 64 male and 64 female birds were divided into 4 groups of 16 replicates, one male and one female per replicate. After a pre-treatment period of two weeks to ensure the birds were already in egg production, the groups were exposed for 6 weeks to 0 (control), 100, 333 or 1000 ppm technical MCPP-P DMA. The test substance contained 765.7 g/l mecoprop-P DMA and the density was 1.137 g/ml. The test substance was analysed in the diet to vary less than 11% from nominal values. The eggs laid were incubated to hatching and chicks observed over a 14-day period.

Results

At termination of the 6 week exposure period, the female groups mean bodyweight in all treatment groups were significantly higher compared to control ($p < 0.05$). This was not considered a substance related effect. Food consumption was not affected by treatment. At 1000 ppm, oviduct weight was decreased ($p < 0.05$) but apparently had no effect on health or the birds' reproductive performance.

Of the reproductive variables, statistical analyses showed no significant treatment related effects ($p < 0.05$). The observations include:

The number of eggs laid per female was slightly lower in 1000 ppm group.

The proportion of cracked eggs laid was slightly higher in the 333 ppm group than in other groups.

Mean egg shell thickness was similar in all groups.

Mean egg weight was similar in all groups.

The proportion of viable embryos of eggs set was similar in all groups.

The proportion of normal hatchlings of eggs set and of viable embryos was similar in all groups.

The number of 14-day survivors of hatchlings and hatchlings per female showed a slight decrease in the 333 and 1000 ppm groups.

Chick bodyweights at hatching and after 14 days were similar in all groups.

It was concluded that dietary administration of up to 1000 ppm technical mecoprop-P DMA had no adverse effect on health and reproductive performance of adult Japanese quails or on the health or growth of their chicks.

The NOEL for reproduction was considered to be 1000 ppm technical MCPP-P DMA.

Table B.9.1.1-09: Summary of the reproductive data

Variable	Dietary concentration (ppm)			
	0	100	333	1000
Technical MCPP-P DMA salt, nominal	0	100	333	1000
Pure MCPP-P DMA salt ¹⁾ , nominal	0	67.3	224	673
Adult birds				
Mean bodyweight at –2 and after 6 weeks (male, g)	182 / 190	183 / 194	176 / 188	180 / 189
Mean bodyweight at –2 and after 6 weeks (female, g)	219 / 216	224 / 231	220 / 226	210 / 218
Mean food consumption during exposure (g/bird/day)	27	28	27	27
Reproduction				
Eggs laid, total number	594	651	651	617
Eggs laid per female	40.7 ²⁾	40.6	40.7	38.5
Eggs cracked, number	38	46	60	43
Eggs cracked, % of eggs laid	6.4	7.1	9.2	7.0
Egg shell thickness, mean (mm)	0.20	0.20	0.21	0.20
Egg weight, mean (g)	11.4	11.8	11.6	11.3
Eggs set	512	559	550	533
Viable embryos	498	531	540	496
Viable embryos of eggs set (%)	97	95	98	93
Hatchlings, number	491	518	523	478
Hatchlings of eggs set (%)	96	93	95	90
Hatchlings of viable embryos (%)	99	98	97	96
14-day survivors, number	484	507	491	465
14-day survivors of eggs laid (%)	81	78	75	75
14-day survivors of eggs set (%)	94.5	90.7	89.3	87.2
14-day survivors of hatchlings (%)	99	98	94	97
14-day survivors per female, number	33.1	31.9	30.7	29.0
Chick bodyweight at hatching (g)	8.0	8.2	8.1	7.8
Chick bodyweight at 14 day (g)	56	57	56	57

¹⁾ Pure MCPP-P DMA = tech. MCPP-P DMA*0,7657/1.137.

²⁾ Please see comments below.

RMS Comments

The number of 14-day old survivors per hen per day is the integrated biological endpoint of this test. The test performed under this guideline begins with birds that are already in egg production. Parameters for adult toxicity and for reproduction are evaluated by making statistical comparisons between treated groups and the control group.

Two mortalities in the control group were observed. They were not explained and the birds were not replaced. At least sixteen breeding pairs of control birds that have produced eggs must be available at the end of the 6-week treatment period according to the guideline validity criteria. The two mortalities occurred in the 3rd week which means that the control group consisted of only 90 “breeding weeks” against 96 (16*6) in the exposure groups. This was not considered in the calculations where the number 40.7 eggs laid per female is erroneous and should be replaced with 39.6 eggs per female.

The no observed effect concentration (NOEC) was determined for adult health and reproductive parameters to be 1000 ppm technical MCPP-P DMA salt equivalent to 673 ppm pure MCPP-P DMA salt.

The NOEC concentration of the test substance in the diet is expressed as mg/kg of diet. The concentration should also be expressed as mg/kg body weight per bird per day. This was not done.

However, the typical values for reproductive parameters are within the values presented in the guideline and no significant effects were observed. Therefore the study is considered valid and the result acceptable.

The NOEC value was not stated for MCPP-P free acid, but based on the molecular ratio between MCPP-P DMA and MCPP-P (259.72/214.65), RMS has calculated the NOEC for MCPP-P to 556 ppm.

RMS comments (renewal):

The RMS has revisited the study report for the purpose of active substance review and judges it to still be valid to demonstrate the long-term toxicity of mecoprop-p DMA to the Japanese quail. The study was conducted in good general adherence with the OPPTS guideline 850.2300 (1996 version). In accordance with the conclusion of Addendum II to the original DAR (July 2002), the NOEC in terms of mecoprop-P is 556 mg a.s./kg food. As only 3 concentrations were tested it is considered that the study is not appropriate to derive a robust LC₁₀ and LC₂₀ endpoint.

For use in a modern avian risk assessment this endpoint requires conversion to be expressed in terms of mg a.s./kg bodyweight/day. For the nominal dose group 673 ppm (as DMA salt, used to set the NOEC in the study), the average body weight reported in the study was 184.5 g (males) and 214 g (females). Mean weekly food consumption for this group throughout the dose period was 27.3 g/bird/day. As such the daily dietary dose for use in the current risk assessment scheme can be calculated to be 99.6 mg/kg bw/day (males) and for females = 85.8 mg/kg bw/day as DMA salt. The equivalent endpoints as MCP-P (acid) are calculated to be 82.3 mg a.s./kg bw/day (males) and 70.9 mg a.s./kg bw/day (females). The lower of these values should be utilised in any risk assessment.

B.9.1.2. Effects on terrestrial vertebrates other than birds**B.9.1.2.1. Acute oral toxicity to mammals**

All relevant studies are provided in Volume 3 – B.6 of the Review Assessment Report.

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

All relevant studies are provided in Volume 3 – B.6 of the Review Assessment Report.

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

No studies are provided in support of this data point. Studies on the effects of the active substance bioconcentration in the prey of birds and mammals are not required since the log Pow of mecoprop-P is less than the trigger value of 3

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

No studies are provided in support of this data point.

B.9.1.5. Potential for endocrine disruption

No data was submitted and therefore no conclusions can be drawn. Member States should note that there are currently no defined criteria for identifying endocrine disruptors under 1107/2009.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

Ref.: IIA. 8.2.1. Bogers, 1990 a: MCPP (as DMA salt) 96 hour acute toxicity study (LC50) in the carp (static).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The toxicity of MCPP (as DMA salt) to fish was studied on carp (*Cyprinus carpio*) in a 96 hour static study according to OECD 203 and EEC Dir 84/449, C.1.

MCPP, 91.6% pure was used in the nominal concentrations 100, 180, 320, 560 and 1000 mg/l based on the amount of MCPP saturated with dimethylamine (DMA) added to the test media. The concentrations were verified by analysis by HPLC. Ten fish with a mean length of 3.1 cm and mean weight of 0.43 g were exposed to each concentration in 4 l test medium, resulting in a loading of about 1 g fish/l. In the test media, pH ranged from 7.1 to 8.6 and the temperature was about 22°C.

Results

LC₅₀ (96h) was determined to be between 320 and 560 mg/l. After 96 hours, 100% mortality in 1000 mg/l and 90% mortality in 560 mg/l were observed. Effects such as hyperactivity at 320 mg/l and discoloration at 560 mg/l were observed. NOEC was determined to be 180 mg/l.

The measured concentrations at the study initiation were 89%, 91% and 94% relative to the nominal 100, 320 and 1000 mg/l, respectively. The actual concentration remained >80% during the exposure period.

Table B.9.2.1-01: Results

Concentration, mg/l		% mortality after exposure period (hours)			
Nominal	Measured	24	48	72	96
0	0	0	0	0	0
100	89	0	0	0	0
180		0	0	0	0
320	291	0	0	0	0
560		0	10	20	90
1000	897	0	100	100	100

RMS Comments

There was only one vessel per concentration. The missing replication reduces the validity of the study.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance. Studies on racemic material (with which the above study was conducted) were previously evaluated but are now disregarded as there is sufficient data on mecoprop-P for this evaluation.

Ref.: IIA. 8.2.1. Bogers, 1990 b: MCP (as DMA salt) 96 hour acute toxicity study (LC50) in the rainbow trout (flow through).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The toxicity of MCP (as DMA salt) to fish was studied on rainbow trout (*Salmo gairdneri*) in a 96 hour flow through study according to OECD 203 and EEC Dir 84/449, C-1.

MCP, 91.6% pure were used in the nominal concentrations 100, 180, 320, 560 and 1000 mg/l based on the amount of MCP saturated with dimethylamine (DMA) added to the test media. The test concentrations were verified by chemical analysis. Ten fish with a mean length of 6.89 cm and mean weight of 3.73 g were exposed to each concentration in 30 l aquaria with a flow rate of 6 l per hour. In the test media, pH ranged from 7.85 to 8.26 and the temperature varied between 15 to 17°C.

Results

LC₅₀ (96h) was determined to be 240 mg/l (95% confidence interval 180-320 mg/l). After 96 hours, 100% mortality in 320-1000 mg/l was observed. Effects on behaviour and discolouration at 100-180 mg/l were observed. NOEC was determined to be <100 mg/l.

The measured concentrations during the study were generally above 80% and therefore the results are based on nominal values.

Table B.9.2.1-02: Mortality

Concentration, mg/l		% mortality after exposure period (hours)			
Nominal	Mean measured	24	48	72	96
0	0	0	0	0	0
100	92	0	0	0	0
180	174	0	0	0	0
320	282	0	50	100	100
560		100	100	100	100
1000	1000	100	100	100	100

RMS Comments

The study is acceptable.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance. Studies on racemic material (with which the above study was conducted) were previously evaluated but are now disregarded as there is sufficient data on mecoprop-P for this evaluation.

Ref.: IIA. 8.2.1. Munk, 1984 a: Report on the study of the acute toxicity of CMPP (Mecoprop) - racemate to Rainbow trout (*Salmo gairdneri*).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of mecoprop (racemate, 92.7% pure) to fish was studied on rainbow trout (*Salmo gairdneri*) according to OECD 203 using a static procedure.

The test substance was added to the aquarium without pre-treatment and stirred. The fish had an average body length of 4.7 cm and an average body weight of 1.0 g. Ten fish were used per test concentration which were nominally 0, 31.6, 46.6, 68.1, 100, 147, 215 and 316 mg/l. The study was performed in aquaria with the loading 0.2 g fish/l water, the temperature 17°C, pH 8 and an oxygen content of 8.9-9.3 mg O₂/l.

Undissolved test substance was observed in all concentrations and the amount increased with increasing concentration. After 72 hours, no undissolved test substance was visible.

Results

LD₅₀ was not calculated but determined to be between 147 and 215 mg/l. All fish died at 215 mg/l after 24 hours and after 48 hours 1 fish (10%) died at 147 mg/l. After 24 hours, symptoms of toxicity such as reduced escape reflex and convulsions were observed at 100 and 147 mg/l. NOEC was determined to be 68 mg/l.

RMS Comments

The substance is not fully dissolved before 24 to 48 hours according to the measurements. The substance should be dissolved before added to the test media. This reduces the reliability of the study.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance. Based on the above original RMS summary however, there were issues with full incorporation of the test item into the media, and no confirmation of achieved organism exposures. As such the study is not considered reliable and is not used for the purposes of renewal.

Ref.: IIA. 8.2.1. Munk, 1984 b.: Report on the study of the acute toxicity of CMPP (Mecoprop) - D-form to Rainbow trout (*Salmo gairdneri*). Report no. BASF 84/10031

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of MCPP (Mecoprop) - D-form (reg. No. 154241) = MCPP-P, 98.6% pure, to fish was studied on Rainbow trout (*Salmo gairdneri*) according to OECD 203 using a static procedure.

The test substance was added to the aquarium without pre-treatment and stirred. The fish had the average body length of 4.7 cm and average body weight of 1.0 g. Ten fish were used per test concentration which was nominally 0, 31.6, 46.4, 68.1, 100, 147, 215 and 316 mg/l. The study was performed in aquaria with the loading 0.2 g fish/l water, the temperature 17°C, pH 8 and oxygen content 9.1-10.1 mg O₂/l.

Undissolved test substance was seen in all concentrations and increased with increasing concentration. After 72 hours, no undissolved test substance was visible.

Results

LD₅₀ was not calculated but determined to be between 147 and 215 mg/l. All fish died at 215 mg/l after 4 hours, and after 1 hour at 316 mg/l. After 24 hours, symptoms of toxicity such as discolouration, reduced escape reflexes and convulsions were observed at 100 and 147 mg/l. NOEC was determined to be 68.1 mg/l.

RMS Comments

The substance is not fully dissolved before 24 to 48 hours according to the measurements. This reduces the reliability of the study.

RMS comments (renewal):

The RMS has revisited the study report for the purpose of renewal of the active substance mecoprop-P. Validity criteria were met in accordance with OECD guideline no. 203 (1984), as control mortality was 0% and dissolved oxygen was retained above 60% saturation (9.1 mg/L equivalent to approximately 94% at 17°C). It is noted that analytical confirmation of the test item showed that nominal concentrations \pm 20% were not achieved. The measured concentrations reported for the study were as follows:

Table B.9.2.1-03: measured concentrations

NOMINAL CONC. (MG/L)
31.6
46.4
68.1
100.0
147.0
215.0
316.0

As such the geometric mean measured concentrations should be used to derive any endpoint from the study. The geometric mean measured concentration at the highest group at which no mortality was observed (nominally 147 mg/L) is 135 mg/L. The next tested concentration (nominally 215 mg/L) resulted in 100% fish mortality in the study, and the geometric mean measured concentration is calculated to be 207 mg/L. As such the LC₅₀ can be estimated to be the midpoint between these two mean measured exposures.

The LC₅₀ from the study is therefore confirmed to be 171 mg a.s./L as mecoprop-P

Ref.: IIA. 8.2.1. Munk, 1986 c: Testing for acute toxicity in fish. MCP (Mecoprop) to the Bluegill sunfish (Lepomis macrochirus).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of Mecoprop (racemate, 92.7% pure) was studied on Bluegill sunfish (*Lepomis macrochirus*) according to US-EPA Subdivision E 72-1 using a static procedure.

The test substance was added to 1 l, stirred and then transferring it to the aquarium. The fish had an average body length of 4.2 cm and average body weight of 0.9 g. Ten fish were used per test concentration which was nominally 0, 21.5, 31.6, 46.6, 68.1 and 100 mg/l. The study was performed in aquaria with the loading about 0.2 g fish/l water, the temperature 23°C, pH 8.1 and the oxygen content 8.2 mg O₂/l.

Undissolved test substance was seen in the aquaria after 24 hours. After 96 hours, no undissolved test substance was visible.

Results

No mortality and no signs of toxicity were observed. NOEC was determined to be ≥ 100 mg/l.

RMS Comments

The test substance should be dissolved before adding to the test media according to guideline. This reduces the reliability of the test result.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance. Based on the above original RMS summary however, there were issues with full incorporation of the test item into the media, and no confirmation of achieved organism exposures. As such the study is not considered reliable and is not used for the purposes of renewal.

Ref.: IIA. 8.2.1. Munk, 1989: Report on the study of the acute toxicity of mecoprop-P. Bluegill (*Lepomis macrochirus*).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of mecoprop-P acid (91.4% pure) was studied on Bluegill sunfish (*Lepomis macrochirus*) according to US-EPA Subdivision E 72-1 using a static procedure.

The test substance was added to 1 l, stirred and then transferred to the aquarium. The fish had an average body length of 5.7 cm and an average body weight of 2.1 g. Ten fish were used per test concentration which was nominally 0, 50 and 100 mg/l. The concentration 100 mg/l was performed in triplicate i.e. 3x10 fish. The study was performed in 10 l aquaria with the loading of about 0.2 g fish/l water, the temperature 22°C, pH 8 and an oxygen content of 8.3-7.9 mg O₂/l.

Results

One to two fish died in the 3 aquaria containing 100 mg/l (13% mortality) and toxic symptoms like apathy, convulsions, narcotic like state and tumbling was observed during 4-72 hours. No mortality and no signs of toxicity were observed at 50 mg/l. NOEC was determined to be 50 mg/l.

The measured concentration was 97% of nominal.

RMS Comments

The study was acceptable and showed that MCP-P was of low toxicity to Bluegill sunfish.

RMS comments (renewal):

The RMS has revisited the study report under the renewal of the active substance mecoprop-P. The study is considered to be valid and acceptable for regulatory purposes. Control mortality was < 10% after 96 hours, dissolved oxygen was maintained above 60% and the tested concentrations were confirmed as within 80-120% nominal throughout the study duration.

The agreed endpoint is a 96 hour LC₅₀ > 100 mg a.s./L

Ref.: IIIA. 10.2.1. Kirsch & Munk, 1992 a: Report on the study of the acute toxicity of mecoprop-P DMA salt on rainbow trout (*Oncorhynchus mykiss*). Report no. 92/11938

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The toxicity of the mecoprop-P DMA salt to fish was studied on rainbow trout (*Oncorhynchus mykiss*) in a 96 hour static study according to OECD 203 and 84/449/EEC, C-1.

MCP-P DMA salt with a purity of 746.8 g/l as DMA salt (617 g/l as acid) were used in the nominal concentrations 0, 100 and 150 mg MCP-P DMA/l corresponding to 0, 74.7 and 112 mg a.s./L. Ten fish with a mean length of 6.09 cm and mean weight of 2.43 g were exposed to each concentration in 100 l aquaria. 150 mg/l was studied in triplicate. In the test media, pH was 8.5 and the temperature was 12°C.

Results

No mortality or symptoms of toxicity were observed at any concentration. LC₅₀ (96h) was determined to be >150 mg MCP-P DMA/l. NOEC was determined to be 150 mg MCP-P DMA/l.

The mean measured concentrations during the study varied between 97.5% and 100.7% and therefore the results are based on nominal values.

RMS Comments

The study is performed according to the guideline and is acceptable. Apparently, a formulation is used resembling Duplosan KV. If this is the case, NOEC (96h) calculated as MCP-P DMA is 112 mg/l and when calculating based on the acid content NOEC (96h) is 93 mg/l mecoprop-P acid.

RMS comments (renewal):

The study report has been revisited by the RMS for the purposes of active substance renewal. The study is considered to be conducted in good adherence with OECD guideline no.203. Measured concentrations were maintained within 80-120% of nominal. The study endpoint is therefore confirmed to be an LC₅₀ > 112 mg/L as MCP-P DMA, or > 93 mg/l mecoprop-P acid.

Ref.: IIIA. 10.2.1. Kirsch & Munk, 1992 b: Study report. Acute toxicity study on the Bluegill (*Lepomis macrochirus* RAF) of mecoprop-P DMA salt in a static system (96hours). Report number 92/11941

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of mecoprop-P DMA salt (746.8 g/l purity) was studied on Bluegill sunfish (*Lepomis macrochirus*) using a static 96 hour test according to US-EPA Subdivision E 72-1, 84/449/EEC C1 and OECD 203.

The test substance was added to 1 l, stirred and then transferred to the aquarium. The fish had an average body length of 5.0 cm and average body weight of 1.96 g. Ten fish were used per test concentration which was nominally 0, 100 and 150 mg/l. The concentration 150 mg/l was performed in triplicate. The study was performed in 100 l aquaria with the loading about 0.2 g fish/l water, the temperature 22°C, pH 8.5 and an oxygen content of 8.3 mg O₂/l initially.

Results

No mortality and no signs of toxicity were observed at 150 mg/l. LC₅₀ was then determined to be above 150 mg/l and NOEC 150 mg/l.

The measured concentrations were 99.7-100.1% of nominal.

RMS Comments

The study was acceptable and showed that MCP-P DMA salt was of low toxicity to Bluegill. The oxygen content fell drastically at the end of study and aeration was initiated but as no mortality occurred it is considered of no significant importance to the result.

As in the study on rainbow, apparently, a formulation is used resembling Duplosan KV. If this is the case, NOEC (96h) calculated as MCP-P DMA is 112 mg/l and when calculating based on the acid content NOEC (96h) is 93 mg/l mecoprop-P acid.

RMS comments (renewal):

The study report has been revisited by the RMS for the purposes of active substance renewal. The study endpoint is therefore confirmed to be an $LC_{50} > 112$ mg/L as MCP-P DMA, or > 93 mg/l mecoprop-P acid. It is noted that not all vessels had a dissolved oxygen content maintained above 60%, which is a validity criteria according to the referenced study guideline. However, as this did not appear to cause increased toxic symptoms to fish in those vessels, the RMS still considers the study endpoint to be reliable.

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

Ref.: IIA. 8.2.2. Bogers, 1990 c: MCP-P (as DMA salt) 21-day prolonged toxicity study in the rainbow trout (flow through).

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The prolonged toxicity of MCP-P (as DMA salt) to fish was studied on rainbow trout (*Salmo gairdneri*) in a 21 day flow through study according to OECD 204.

MCP-P, 91.6% pure were used in the nominal concentrations 4.8, 10, 23, 48 and 100 mg/l based on the amount of MCP-P added to test media saturated with dimethylamine (DMA). Ten fish with a mean length of 6.0 cm and mean weight of 3.1 g were exposed to each concentration in 30 l aquaria with a flow rate of 6 l per hour. In the test media, pH ranged from 7.8 to 8.1 and the temperature varied from 13 to 15°C.

Results

No mortality or other effects were observed during the study period at any concentration. NOEC for rainbow fish exposed to MCP-P as DMA salt was 108.5 mg/l (± 10.8) based on mean measured values.

RMS Comments

The study was acceptable.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance, nor could the endpoint expressed in terms of mecoprop-P (due to no confirmed content of the active substance within the DMA salt). It should be noted that this study design is no longer considered suitable to detect true sub-lethal effects on fish (see section 8.2.2 of EU 283/2013).

Ref.: IIA. 8.2.2. Munk, 1993: Sublethal toxic effects on the rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) of mecoprop-P-acid in a flow through system (28 days); OECD 204.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

Sublethal toxic effects on the rainbow trout (*Oncorhynchus mykiss*) of Mecoprop-P-acid, 92.7% pure, was studied in a flow through system during 28 days according to OECD 204.

Twenty fish per test group were exposed to the concentrations 0, 1, 10, 50 and 100 mg/l in a flow through system. The aquaria contained about 60 l and the water exchange period was 6 h/tank with a flow rate of 10 l/h. The water pH was 8.4, the temperature was 16°C, and the photoperiod 16 h light/day. The mean length of the fish was 6.0 cm and the mean weight was 1.9 g at the beginning of the test. The measured concentrations averaged 96% of the range 88.8-105.1%).

Results

No mortality occurred in any test group. Signs or symptoms of toxicity were only observed in the highest test group of 100 mg/l where slight discoloration was observed on day 2 and 5 of the study. Compared with the control group at the end of the study, the mean body weight at 100 mg/l was statistically significant smaller ($p \leq 0.05$) and the mean body length at 100 mg/l was statistically significant smaller ($p \leq 0.01$). NOEC was 50 mg/l.

Table B.9.2.2-01: Effect results

Effect variable	Nominal concentration (mg/l)				
	0	1	10	50	100
Mortality %	0	0	0	0	0
Body weight					
day 0	1.89 ±0.300	1.89 ±0.248	1.92 ±0.260	1.95 ±0.263	2.01 ±0.309
day 28	6.64 ±0.998	6.48 ±0.702	6.66 ±0.940	6.42 ±0.988	5.89 ±0.972
Body length					
day 0	6.01 ±0.327	5.99 ±0.354	6.07 ±0.326	6.07 ±0.225	6.06 ±0.295
day 28	8.55 ±0.415	8.55 ±0.405	8.66 ±0.452	8.41 ±0.444	8.00 ±0.486

RMS Comments

The study was acceptable.

RMS comments (renewal):

The RMS has not revisited this study for the purposes of renewal. From the original RMS summary the 21-day adult NOEC is confirmed as 50 mg a.s./L. It should be noted that this study design is no longer considered suitable to detect true sub-lethal effects on fish (see section 8.2.2 of EU 283/2013).

Report:	CA 8.2.2.1/01, [REDACTED] (2015)
Title	Mecoprop-P acid: Toxic effects to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in an Early-Life Stage toxicity test Report No. D92378
Guidelines:	OECD 210 (2013)
GLP:	Yes
Deviations	none

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

1. **Test materials:** Mecoprop-P acid, purity 94.62%
Description: Pale yellow solid
Lot/Batch #: 3860
2. **Vehicle/solvent control:** 3,4 dimethylformamide analytical grade (DMF)
3. **Test animals**
Species: Rainbow trout, *Oncorhynchus mykiss*
Source: [REDACTED]
Acclimatisation period: Prior to test start the eggs were acclimated to the test water for about 30 minutes.
Feed: During embryo-stage and for newly hatched larvae no food was supplied. After the yolk sac had been consumed by some of the fish, food was provided to the fish at least twice each working day. The fish were fed with a commercial trout fish food (HOKOVIT, supplied by H.U. Hoffmann AG, CH-4922 Bützberg, Switzerland). Food was given to the juvenile fish *ad libitum* while minimizing the surplus. On weekends, food was given twice per day.
4. **Environmental conditions**
Temperature: Vessel water temperature ranged 11.5 to 12.3°C
Photoperiod: The eggs were kept in complete darkness until Day 29. Thereafter a 16 hour light to 8 hour dark photoperiod, with a 30 minute transition period was used.
Light intensity during the light period ranged 150 to 280 Lux.
Test water: Local tap water (non chlorinated well water of drinking water quality), reduced for total hardness by ion exchange. Total hardness of test water: 214-232 mg/L as CaCO₃. The test water was aerated prior to the preparation of the test media.

B. STUDY DESIGN AND METHODS

1. In life dates:

11/11/2014 – 29/04/2015

2. Test system:

Four replicate 16 liter flow-through glass aquaria (length: 25 cm; wide: 18 cm; height: 35 cm) containing approximately 13 liters of test medium (height of water level: approx. 29 cm) were used for each test concentration, the control and the solvent control. The glass aquaria were positioned in temperature regulated water baths (two replicates in one water bath). For incubation of the eggs, in each of the replicate aquaria, a stainless steel egg cup (8.5 cm in diameter, 8.0 cm in height) with a stainless steel net bottom was included.

3. Test concentration:

The following nominal concentrations were tested: 0 (water control), 0 (DMF solvent control), 0.12, 0.38, 1.2, 3.8, and 12 mg Mecoprop-P/L.

4. Dosing:

Concentrations of the test item in the test media were maintained by dosing application solutions into the test water using an automatic dosing system. The application solutions were regularly renewed during the test period.

Test water flowed at a constant into the mixing vessels, where the test water and application solutions were continuously mixed together using intensive stirring with a magnetic stirrer to prepare test media at the desired nominal concentrations. From the mixing vessel, the test media flowed into

electronically regulated splitting devices and were divided into four identical volumes. These volumes were directed into the corresponding four replicate test vessels of each treatment.

Into each replicate test vessel, the test medium continuously flowed at a rate of 78 liters/day. The volume of the beakers used was 13 liters. Thus, the flow rate of the test media through each of the four replicate glass beakers corresponded to a 6-fold theoretical volume exchange per day.

For preparation of the solvent control, DMF only was dosed into test water in the same way as described above. Test water without any additions flowed at the same rate into the replicates of the control.

5. Experimental treatment:

Four replicates were used for each treatment. At the start of the test (Day 0), 80 fertilized eggs were randomly distributed to the replicate test vessels of each treatment (20 eggs per replicate). The loading rate of the eggs (biomass per volume of test medium in each test vessel) was distinctively lower than 0.5 g/liter as requested by the test guideline.

A few days after complete hatching of the larvae, the incubation cups were removed (Day 34 post fertilization) and the larvae were released into the corresponding 13 liter flow through aquaria without exposing the larvae to the air.

The loading rate (based on the volume of the aquaria) of the test fish at the end of the test period was calculated to be a maximum of 1.5 g fish wet weight/liter. The loading rate (based on the water flow of 78 L/day per replicate) was calculated to be a maximum of 0.25 g fish wet weight/liter/day, thus fulfilling the requirement of the test guideline.

6. Parameters measured:

Hatching rate, development rate, survival of larvae and juvenile fish, fish length and weight.

7. Analysis of test item concentration:

From the freshly prepared application solutions, duplicate samples were taken on three preparation dates (Days -2, 26, and 48). To confirm the stability of the test item in the application solutions during their renewal periods, duplicate samples were taken from the aged application solutions on Days 5, 33 and 56, corresponding to renewal periods of seven and eight days.

From the test media, duplicate 10 mL samples were taken from all test concentrations and the controls at 26 dates starting from Day 0 until test end.

Only the highest test concentration of nominal 12 mg/L was analytically verified, as this test concentration was determined to be the NOEC in this test. From this test concentration, one of the duplicate samples from all sampling times was analyzed.

The samples from all lower test concentrations were only analysed for verification of the dosing system (one sample from nominal 0.12 to 3.8 mg/L on Days 0, 5, and 7).

8. Water quality:

Dissolved oxygen concentration, pH, and water temperature were measured in all replicates from all test concentrations and in the controls at the start and end of the test and twice weekly during the test period.

The total hardness of the test water was measured on 14 dates during the test period.

The appearance of the test media was checked each working day. As no visible abnormalities were observed, observations were recorded once a week.

II. RESULTS AND DISCUSSION

1. Hatching rate:

In all test solutions the first larvae were observed on Day 25 and hatching was completed on Day 29 post fertilization. At all test item concentrations the hatching rate was nearly equal to or even slightly higher compared to the solvent control (range: 97 to 104% of the solvent control value). No concentration-effect relationship was observed.

2. Development rate of eggs:

In the control and at all test concentrations up to and including 11.2 mg/L (12 mg/L nominal), the development rate of the larvae from fertilization to hatching was in the range of 91.0 to 118.4% of the solvent control value. No concentration-effect relationship was observed over the whole concentration range.

3. Survival of larvae and juvenile fish:

The mean survival rate of the fish was $87.1 \pm 8.8\%$ in the control and $88.5 \pm 7.9\%$ in the solvent control, demonstrating the suitability of the test conditions (the validity limit for post hatch success is $\geq 75\%$).

The mean survival rates at the test concentrations up to and including 11.2 mg/L (12 mg/L nominal) were in the range of $82.0 \pm 6.4\%$ to $90.3 \pm 2.5\%$. These values were nearly identical or even slightly higher compared to the solvent control and no concentration effect relationship was observed. All fish, which survived until the end of the test were healthy and showed normal behavior.

4. Fish length and body weight:

The mean measured body length, body wet weight and body dry weight of the test fish obtained from all test concentrations up to and including 11.2 mg/L were equal or even slightly higher compared to the solvent control.

Table B.9.2.2-02: Observed Results of hatching, development, survival, body length and wet weights of *O.mykiss*

Measured parameter	Nominal concentration (mg a.s./L)						
	0 (control)	0 (DMF control)	0.12	0.38	1.2	3.8	12
Hatching (%)	87.5	87.5	90	88.8	85.0	91.3	90.0
Development	0.380	0.407	0.394	0.370	0.482	0.412	0.423
Survival (%)	87.1	88.5	87.5	88.8	82.0	89.5	90.3
Body length (mm) [% DMF control]	47	48	48	48	49	48	49
Wet weight (g) [% DMF control]	1.03	1.03	1.07	1.04	1.11	1.07	1.10

5. Analytical results:

The test item concentrations in the freshly prepared and aged application solution used for the dosage of the highest test concentration of nominal 12 mg/L ranged from 93 to 115% of the nominal value. This shows the correct preparation of the application solution and the stability of the test item in the application solution during its renewal periods of 7 to 8 days.

The analytical measurements from all test concentrations of nominal 0.12 to 12 mg/L during the first seven days of the test (verification of the dosing system) showed recoveries in the range of 81 to 110%

of the nominal values. This shows that the dosage of all test concentration levels used in the test was acceptable.

The test item concentration in the test medium of nominal 12 mg/L (determined as the overall NOEC) varied in the range from 84 to 111% of the nominal value during the whole exposure period of 89 days, except on Days 14 and 76, where slightly lower values of 74 and 68% of nominal were determined. The analytical results demonstrate, that the test concentration was sufficiently maintained during the test period of 89 days.

The arithmetic mean measured concentration of this highest tested concentration was calculated to be 11.2 mg/L, with the geometric mean concentration calculated to be 11.1 mg/L

6. Water quality:

The mean water temperature in the controls and at the different test concentrations over the course of the test was 11.9 °C (range 11.5 to 12.3 °C). The differences in water temperature between the treatments or between successive days at any time during the test did not exceed 0.7°C, and thus, were within the range of ± 1.5 °C, as requested by the test guideline.

During the test period, the dissolved oxygen concentration in the controls and at the test concentrations was at least 7.8 mg/L, corresponding to an oxygen saturation of at least 71% (considering a water temperature of 11-12°C. Thus, dissolved oxygen concentration was sufficiently high throughout the test period, fulfilling the test guideline requirement of a 60%-minimum.

The pH values in the controls and at the test concentrations ranged between 7.8 and 8.1.

The total hardness of the test water was in the range of 214 to 232 mg/L CaCO₃.

No remarkable observations were made concerning the appearance of the media. The test media were clear solutions throughout the test period.

7. Validity criteria:

In accordance with OECD guideline no. 210 (July 2013) the following criteria are required to demonstrate a valid test:

- the dissolved oxygen concentration should be >60% of the air saturation value throughout the test; Minimum achieved = 71%
- the water temperature should not differ by more than 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species; maximum variation = 0.7 °C, range for species = 10 ± 1.5 °C.
- the analytical measure of the test concentrations is compulsory; this was performed on multiple occasions, see analytical section above.
- survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined (for *O.mykiss* = 75%); achieved control and solvent control hatching success = 87.5%. Control and solvent control post-hatch survival = 87.1 and 88.5%, respectively.

III. CONCLUSIONS

Summarizing the NOEC values for each of the test parameters assessed, the overall NOEC of mecoprop-P for early life stages of rainbow trout was determined to be the geometric mean measured test concentration of 11.1 mg/L (nominal 12 mg/L), since no toxic effect on the eggs, larvae or fish was observed up to and including this test concentration. The overall LOEC could not be determined

due to the absence of toxic effect of the test item up to the highest test concentration (LOEC > 11.1 mg/L).

In conclusion, the test item mecoprop-P had no toxic effects to rainbow trout up to the highest test concentration of mean measured 11.1 mg/L.

RMS comments:

The study was well reported and considered to be conducted in good adherence with the modern version of the relevant OECD guideline. There was only one noted deviation, where the mean and maximum achieved temperature in all tested concentrations including both control groups was in excess of the recommended maximum for the species according to OECD 210. However, there were no noted deficiencies in the control group performance so it can be considered that adequate conditions were maintained for normal test organism behaviour. Overall the study is considered to be valid and suitable for use in the risk assessment.

As some analytical measurements at the NOEC concentration were found to be below the nominal range of 80-120%, the endpoint should be based on geometric mean measured concentrations, which means the overall study **NOEC = 11.1 mg mecoprop-P acid/L**. The EC₁₀ and EC₂₀ endpoint values could not be determined due to a lack of evident dose response for the measured parameters.

B.9.2.3. Potential for endocrine disruption

No data was submitted and therefore no conclusions can be drawn. Member States should note that there are currently no defined criteria for identifying endocrine disruptors under 1107/2009.

B.9.2.4. Acute toxicity to aquatic invertebrates

Ref.: IIA. 8.2.4. Bell, 1994: *Mecoprop-P. Acute toxicity to Daphnia magna*. Report no. RNP 447/941082

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of mecoprop-P (89.7% pure) on daphnids was studied on *Daphnia magna* in a 48-hour static test according to EEC Dir 92/69, Part C and OECD 202, part 1.

The daphnids were divided into a control group and 7 exposure groups exposed to the nominal concentrations 0, 1, 2.2, 4.6, 10, 22, 46 and 100 mg/l. Twenty daphnids less than 24 hours old were used in each group in replicates of 10 animals/200 ml test volume. During the study, pH varied in the solutions between 7.0 and 7.8, the oxygen content between 8.2 and 8.4 mg O₂/l, and the temperature was 22°C. The measured concentrations ranged from 81-92% of nominal concentrations.

Results

EC₅₀ (48h) were determined to be > 91 mg/l. NOEC (48h) was determined as > 91 mg/l. Both values are based on the measured concentration.

Table B.9.2.4-01: Test results based on 20 daphnids per concentration

Time	% Daphnids immobilized at nominal concentrations (mg/l)							
	0	1	2.2	4.6	10	22	46	100
24 h	0	0	5	0	0	0	15	5
48 h	0	0	5	0	0	0	15	5

RMS Comments

The study was acceptable. NOEC should have been 91 mg/l in this study.

RMS comments (renewal):

The RMS has revisited the study for the purpose of active substance renewal. The study was conducted in close adherence with OECD guideline no.202, with all related validity criteria met. As such the study is considered to be valid and acceptable. The agreed endpoint is a **48-hr EC₅₀ > 91 mg a.s./L**.

Ref.: IIA. 8.2.4. Elendt-Schneider, 1991: Determination of acute toxicity of mecoprop-P (Reg. No. 154-241) to the water flea Daphnia magna Straus.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of mecoprop-P (>90% pure) on daphnids was studied on *Daphnia magna* in a 48-hour static test according to EEC Dir 79/831, Annex V Part C.

The daphnids were divided into a control group, a solvent control group and 4 exposure groups exposed to the nominal concentrations of 0, 12.5, 25, 59 or 100 mg/l. Twenty daphnids less than 24 hours old were used in each group in 4 replicates of 5 animals/10 ml test volume.

During the study, pH varied in the solutions between 7.29 and 8.0, the oxygen content between 7.86 and 9.44 mg O₂/l, and the temperature between 19.5 and 20.5°C. The measured concentrations of the test substance varied between 97.6 and 103.5% of nominal concentrations.

Results

EC₅₀ (48h) were determined to be > 100 mg/l because the mortality at 100 mg/l was 10%. NOEC (48h) was determined as 100 mg/l.

Table B.9.2.4-02: Test results based on 20 daphnids per concentration

Time	% Daphnids immobilized at nominal concentrations (mg/l)					
	0	SC	12.5	25	50	100
24 h	0	0	0	0	0	0
48 h	5	0	0	0	0	10

SC: solvent control

RMS Comments

The study was acceptable. NOEC may be considered 50 mg/l as 2 out of 20 daphnids died at the highest test level.

RMS comments (renewal):

The RMS has revisited the study for the purpose of active substance renewal. The study was conducted in close adherence with OECD guideline no.202, with all related validity criteria met. As such the study is considered to be valid and acceptable. The agreed endpoint is a **48-hr EC₅₀ > 100 mg a.s./L**.

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

Ref.: IIA. 8.2.5. Dohmen, 1993 a: Effects of Mecoprop-P on the reproduction of Daphnia magna Straus in a chronic toxicity test.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The effects of mecoprop-P on the reproduction of *Daphnia magna* was studied in a 21-day semi static test according to EEC guideline XI/681/86 (draft 4) and in part OECD 202.

The test substance was mecoprop-P (free acid) 92.2% pure. The test medium was prepared on the basis of ultrapure deionized water and during the study period of 21 days it was renewed 9 times.

Based on the acute study by Elandt-Schneider (1990), who found EC_{50} (48h) = 100 mg/l, the following nominal concentrations were used 0, 2.5, 10, 25, 50 and 100 mg/l.

For each concentration and for the control, ten replicates were carried out using one *Daphnia* in each vessel of 50 ml. The test systems were held in an incubator at 21°C under 16 hours light per day at 1000 Lux. The pH was about 8. The measured concentrations varied in the range of 98.9% to 107.6% of the nominal concentration.

Results

NOEC was determined to be 50 mg/l and LOEC to be 100 mg/l.

At 50 mg/l, there seems to be a slight effect on reproduction but only at 100 mg/l a statistically significant effect ($P < 0.05$) on reproduction was observed. At 100 mg/l, besides 43% reduction in number of live offspring, some offspring appeared dead and aborted eggs were observed.

No mortality was observed among adults. The first offspring were seen at day 9 of the study.

Table B.9.2.5-01: Results on the mortality and reproduction

Effect	Results after 21 days of exposure of MCP-P at the nominal concentration (mg/l)						
	0	1.0	2.5	10	25	50	100
Mortality %, adults	0	0	0	0	0	0	0
No. young accumulated	880	930	989	941	992	742	498
No. young/adult \pm SD	88 \pm 16	93 \pm 20	99 \pm 14	94 \pm 9	99 \pm 5	74 \pm 16	50 \pm 14
% of control	100	106	112	107	113	84	57

RMS Comments

The study was acceptable.

RMS comments (renewal):

The RMS has revisited the study for the purpose of active substance renewal. The study was conducted in close adherence with OECD guideline no.211, with all related validity criteria met. As such the study is considered to be valid and acceptable. The agreed endpoint is a 21-day NOEC = 50 mg a.s./L, based on nominal concentrations.

Ref.: IIA. 8.2.5. Müllerschön, 1990: Influence of MCP-P (as DMA salt) on the reproduction of *Daphnia magna*.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The influence on the reproduction of *Daphnia magna* of MCP-P (as DMA salt) about 91.6% pure was studied in a 28 day semi-static study according to OECD 202, section 2.

The test solution was prepared by dissolving MCPP in a dimethylamine (DMA) solution. On the day of the experiment, the DMA salt of the MCPP was diluted into the test medium. MCPP (as DMA salt) were used in the nominal concentrations (calculated as amount of MCPP acid): 2.5, 7.4, 22.2, 66.7 and 200 mg/l. The test mediums were renewed 3 times per week. The daphnids were fed with the algae *Scenedesmus sp.*

The study was performed in glass beakers each containing 200 ml reconstituted water. Ten Daphnia less than 24 hours old were exposed for 4 days in duplicate. The daphnids were then separated each in 80 ml beakers and exposed in 21 days at 20°C and 16 hours of light/day (500-2000 Lux). The pH was 8.0.

Results

NOEC based on inhibited reproduction was determined to be 22.2 mg/l.

A dose-related increase in mortality of adult daphnids was observed for test concentrations 66.7 mg/l and 200 mg/l. After 48 hours, no mortality was observed at any concentration. After 96 hours 25% were dead at 66.7 mg/l and 25% at 200 mg/l using 2x10 daphnids. After the separation in 10 single survivors into brooding beakers (10x1), 20% mortality at 66.7 mg/l and 100% at 200 mg/l were observed after 16 days. The reproduction started after 12 days of exposure.

Table B.9.2.5-02: Results on the mortality and reproduction

Effect	Results after 21 days of exposure of MCPP at the nominal concentration (mg/l)					
	0	2.5	7.4	22.2	66.7	200
Mortality %						
After day 4 n=20	0	0	5	0	25	25
After day 21 n=10	0	0	0	0	20	100
No. young, accumulated	775	1067	912	707	186	0
No. young/adult	78 ±22	107 ±11	91 ±15	71 ±10	19 ±14	0
% of control	100	138	118	91	24	0

RMS Comments

The study is acceptable.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance. The above study (*Dohmen, 1993 a*) is considered adequate to address this data requirement for long-term toxicity testing to *Daphnia*. It is noted however that the NOEC from this study is lower. As such the original RMS-confirmed **NOEC of 22.2 mg a.s./L** will be utilised in the renewal risk assessment for mecoprop-P.

B.9.2.6. Effects on algal growth

Ref.: IIA. 8.2.6. *Dohmen, 1993 b: Effect of mecoprop-P on the growth of the green alga Pseudokirchneriella subcapitata.*

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The effect of mecoprop-P, 92.2% pure, on algae was studied on the green alga *Pseudokirchneriella subcapitata* using a 72-hour growth inhibition test according to OECD 201.

The test was performed at the concentrations 0, 3, 9, 27, 81, 243 and 729 mg/l using a final volume of 60 ml in five replicates each inoculated with algae at an initial cell concentration of about 3×10^4 cells/ml. The culture flasks were placed in an incubator at 23°C at continuous illumination at 8000 Lux and kept in suspension by constant shaking. The cell densities were measured by a photometer.

Results

Using the nominal concentrations the effect levels were obtained by graphic determination:

based on biomass $E_b C_{50} (0-72h) = 270 \text{ mg/l}$
 $NOE_b C (72) = 27 \text{ mg/l}$
 based on growth rate $E_r C_{50} (0-72h) = > 729 \text{ mg/l}$

The mean measured values ranged 97.4 to 105.2% of nominal concentrations.

Table B.9.2.6-01: Results on biomass calculations and growth rates.

	Mean values at the MCPP-P concentration (mg/l)						
	0	3	9	27	81	243	729
Cell density ($\times 10^5$)							
24 h	1.736	1.729	1.798	1.619	1.564	1.248	0.710
48 h	8.492	9.022	9.022	7.866	7.164	4.688	1.715
72 h	25.75	27.60	26.81	23.84	20.61	13.48	5.321
Area	536.5	571.2	563.4	495.7	438.8	286.2	104.1
% inhibition	0	-6.5	-5.0	7.6	18.2	46.7	80.6
Growth rates							
24 h	1.753	1.748	1.790	1.625	1.651	1.422	0.814
48 h	1.588	1.656	1.611	1.579	1.522	1.311	0.929
72 h	1.110	1.117	1.089	1.107	1.054	1.068	1.130
Average growth rate	1.484	1.507	1.498	1.458	1.410	1.268	0.959
% inhibition	0	-1.6	-0.9	1.7	5.0	14.5	35.4

RMS Comments

The study was acceptable.

RMS comments (renewal):

The RMS has revisited the study report in the context of active substance renewal. The study was conducted in good adherence with the OECD 201 guideline (1984 version) and the related validity criterion was met (at least factor 16 increase in control group cell density). As such the study is considered to be valid for use. The agreed endpoints are as follows (based on nominal concentrations):

72-hour $E_b C_{10} = 35 \text{ mg a.s./L}$

72-hour $E_b C_{50} = 270 \text{ mg a.s./L}$

72-hour $E_r C_{10} = 145 \text{ mg a.s./L}$

72-hour $E_r C_{50} > 729 \text{ mg a.s./L}$

Reference

Armstrong K. 2000: Mecoprop-P dimethylamine salt, Alga, growth inhibition test (72 h, EC₅₀).

Inveresk Report no. 17864. Mecoprop-p Task Force, BASF Doc ID 2000/1000259.

Fisher K 1999. Establishment of Methodology for the Analysis of Mecoprop-p Dimethylamine Salt in Aquatic Toxicology Media. Inveresk Report no. 17875. Mecoprop-p Task Force, BASF Doc ID 1999/1003116.

Previous evaluation:	Included in Addendum II to DAR (July 2002) for original a.s. approval
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Method

The effect of Mecoprop-P dimethylamine salt on the freshwater blue-green algae *Anabaena flos-aquae* (strain CCAP 1403/13B) was studied in a 72-hour static growth inhibition test performed according to OECD TG 201, 1984.

The test substance was mecoprop-P dimethylamine (MCP-P DMA) salt with a purity of 76.6% (765.7 g/l) and a density of 1.137 g/cm³. The test flasks were 250 ml Erlenmeyer flasks capped with foil lids. Based on a range finding test the definitive concentrations were set (cf. table below) using three replicates per test concentration and six controls. The test concentrations were measured at the beginning and end of test exposure period, i.e. 0 and 72 h, respectively, as mecoprop-P free acid and recalculated to mecoprop-P dimethylamine salt.

Table B.9.2.6-02: Nominal and measured test concentrations

Nominal concentration						
MCP-P DMA salt product	0	10	25.6	64	160	400
equivalent MCP-P DMA salt	0	6.7	17.2	42.9	107	268
equivalent MCP-P free acid	0	5.6	14.3	35.8	90	224
Mean measured concentration						
MCP-P free acid	0	5.955	14.30	36.17	91.35	227.4
equivalent MCP-P DMA salt	0	7.211	17.31	43.78	110.6	275.3

The medium was prepared according to guideline. The temperature varied within 23.6 to 25.2°C. The illumination was provided by artificial fluorescent tubes at a light intensity of 7610 lux. The algae growth inhibition was measured by cell counting after 24, 48 and 72 hours under microscope and Sedgewick-Rafter (over 4 counts) or Neubauer Counting Chambers depending on cell numbers present.

Results

The results are reported as daily cell concentrations (cells/ml), average specific growth rates (μ/day) and normalised area under growth curves (cells/h/ml). The EC₅₀-values and NOEC were calculated by probit transformation and regression analysis.

Table B.9.2.6-03: Summary of results

	Hours	Measured concentration, mg MCP-P DMA salt					
		0	7.211	17.31	43.78	110.6	275.3
Mean cell number (x 10 ⁴ cells/ml)	0	1	1	1	1	1	1
	24	2.35	2.34	2.10	1.44	0.83	0.03
	48	4.23	3.65	2.29	1.83	0.32	0.02
	72	9.76	9.98	3.78	2.92	0.03	0
% inhibition			-2.3%	61.3%	70.1%	99.7%	100.0%
Growth rate (μ/d)	0-72	0.76	0.76	0.44	0.35	0.0	0.0
% inhibition			0.0%	42.1%	53.9%	100.0%	100.0%
AUC (x 10 ⁵ cells/h/ml)	0-72	2.99	2.83	1.26	0.74	0	0
% inhibition			5.4%	57.9%	75.3%	100.0%	100.0%

Table B.9.2.6-04: Effect results for average specific growth rates and Area under the curve (AUC) based on measured concentrations.

	Growth function	Hour interval	EC50 (mg/l)	NOEC (mg/l)
MCP-P DMA salt	Growth rate	0-72	28.9	7.211
	Area under curve	0-72	19.6	7.211
MCP-P	Growth rate	0-72	23.9	5.956
	Area under curve	0-72	16.2	5.956

According to OECD the 100% inhibition observed in the highest exposure groups were not included in the EC₅₀ estimation. The Pearson chi-squared goodness of fit test statistic was statistically significant at the 1% level for AUC (0-72 h) and the growth inhibition curve for each of the concentration curves. As a result, the EC₅₀ values reported can only be used as indicative of the EC₅₀ as the probit fit to the data was not good, i.e. the data were not adequately spread over 0% to 100%.

RMS Comments

Study was performed according to OECD TG 201, 1984. The medium deviates from the guideline recommended medium. The medium was prepared according to a standard algal receipt recommended by Centre for culture of algae and protozoa (CCAP) used at the same place from where the algae *Anabaena flos-aquae* (strain CCAP 1403/13B) was received. The test medium pH was 7.3 to 7.4 at the beginning of the test and varied between 6.8 and 7.3 after 72 hours, which is within the acceptable limits (1.5 pH units). Test substance was stable during the test period. However, the cell density in the control cultures did not increase by a factor of at least 16 within 3 days as prescribed in the guideline quality criteria. The authors note that the OECD guideline is written for fast growing green algae species and that the blue green algae *Anabaena* generally show slower growth than green algae. The growth rates observed indicate that the growth was exponential in the test period and the study is therefore acceptable, although a prolonged exposure period should have been used. The species is recommended by US-EPA who requires 96 hours exposure period. *Anabaena flos-aquae* may be included in the revised OECD algae test.

RMS comments (renewal):

The RMS has revisited the study report in the context of active substance renewal. The above original RMS conclusions are agreed with. The study is considered acceptable, however it was noted that the dose response curve for EC₅₀ derivation over the 72-hour period was not a good fit to the data, due to 'clustering' of inhibition around 0, 50 or 100% in the tested concentrations. However, judging the calculated EC₅₀ endpoints against the observed cell density inhibitions seen in the study the RMS is satisfied that the reported endpoints are indicative of the effects seen in the study.

The agreed endpoints are as follows (based on mean measured concentrations of DMA salt):

72-hour ErC ₅₀ = 28.9 mg/L as DMA salt	23.9 mg/L as MCP-P
72-hour EbC ₅₀ = 19.6 mg/L as DMA salt	16.2 mg/L as MCP-P

Report:	CA 8.2.6.2/01, Jenkins, C.A. (2007)
Title	Mecoprop-P (DMA salt) algal growth inhibition assay <i>Navicula</i> Report No. ZZF0001/063120
Guidelines:	EEC C3 OECD 201(1984)
GLP:	Yes
Deviations	No major deviations

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

1. **Test materials:** Mecoprop-P (DMA salt)
Description: Clear brown liquid
Lot/Batch #: 06/23
Purity: 601.4 g Mecoprop-P/L
CAS #: 66423-09-4
Stability of test compound: Stable
Density: 1.1337 g/mL
2. **Test animals**
Species: *Navicula pelliculosa* (freshwater diatom)
Strain: 1050-3
Source: Sammlung Von Algenkulturen (SAG), The University of Göttingen, Germany
Culture medium: Sterile algal nutrient medium, supplemented with sodium metasilicate solutions
Test vessel: 250 mL conical flasks closed with foam bung, glass beads included to prevent cell clumping
3. **Environmental conditions**
Temperature: 22.2 – 23.1°C
pH: 7.24 – 7.90
Light intensity: 24-hr lighting at 4080 - 4810 Lux

B. STUDY DESIGN AND METHODS

1. Experimental dates:

21 April – 21 June 2006

2. Dose selection rationale

A range finding study was run at concentrations of 1, 10 and 100 mg a.s./L. The definitive test concentrations were based on the results of the range finding test and were nominal concentrations of 0, 6.25, 12.5, 25, 50, 100 and 200 mg Mecoprop-P/L, with 6 control replicates and 3 replicates per tested concentration.

3. Preparation of dosing mixtures

The test medium was prepared by adding the test substance (11.8, 23.6, 47.1, 94.3, 188.5 or 377 mg) to 1000 mL of algal culture medium (according to appendix 2 of OECD 201). An aliquot (13.1 mL) of secondary algal inoculum was added to 500 mL of the test medium at each concentration to give an initial cell density of 1×10^4 cells/mL.

100 mL of inoculated test medium was added to sterilised 250 mL conical flasks. 25 glass beads were added to each flask to reduce clumping of the algal cells. The flasks were then treated by ultra-sound for approximately 5 seconds, again to reduce clumping.

Control cultures were prepared as per the test cultures, except that no test substance was added and a larger volume of medium (800 mL) was made.

4. Measurement of growth

Samples were taken from control and test flasks at 24, 48, 72 and 96 hours and the cell densities were measured using a haemocytometer. The estimate of diatom cell numbers in each sample was based on two counts, each comprising eight haemocytometer cells. The presence of any abnormal cells was also noted during counting.

5. Algistatic/algicidal extension

At the end of the definitive test, aliquots were taken from the highest concentration level, where growth had been severely inhibited and from the controls. A fresh, sterile culture medium was inoculated and the flasks were incubated for 5 days.

6. Statistics

Data were compiled in an Excel spreadsheet and analysed using the Curve Fit application of SAS 8.2 (SAS Institute 1999).

Non-linear regression was used to fit sigmoidal curves to the Area Under the Curve (AUC) and growth rate data.

95% confidence intervals for EC_{50} were calculated using the likelihood ratio method (Donaldson and Schnabel, 1985). The EC_{10} and EC_{20} (with 95% confidence interval) were also estimated by reparameterising the formulae used to calculate the EC_{50} .

For AUC and growth rate, Williams' test (1971, 1972) was also used to compare each treated group with the control unless there was evidence of a non-monotonic dose response relationship, in which case Dunnett's test (1955, 1964) was used.

II. RESULTS AND DISCUSSION

1. Analytical results

The test concentrations were measured using mass spectrometry. At 0 hours duplicate samples were taken from each freshly prepared test media. At 96 hours replicate test vessel media was pooled per treatment and samples taken for analysis. 0 hour measured concentrations (as mecoprop-P) were in the range 81-92% of nominal concentrations and 96-hour old samples were in the range 83-97% of nominal. Results are conservatively based on mean measured concentrations of mecoprop-P.

2. Observations

No microscopic abnormalities of the cells were detected.

3. Algal growth

Table B.9.2.6-05: Cell Densities and average growth rates after 72 and 96 hours

Nominal concentration as mecoprop-P (mg a.s./L)	Measured concentration as mecoprop-P (mg a.s./L)	Cell density (1 x 10 ⁴ cells/mL)		Inhibition of growth rate (%)	
		72-hr	96-hr	72-hr	96-hr
Control	0	40.3	84.8	--	--
6.25	5.93	44.9	93.8	-9.6	-7.7
12.5	10.3	40.7	91.1	-3.2	-4.1
25	21.1	35.2	82.9	1.0	-1.9
50	41.8	23.1	75.3	9.6*	-2.5
100	86.5	11.5	66.6	33.2*	3.4*
200	168	0.729	3.44	109.3*	70.9*

The E_bC_{50} and E_yC_{50} are presented below. The results are expressed in terms of mean-measured levels of mecoprop-P. The 72h result is taken as the endpoint for the EU.

Table B.9.2.6-06: Percentage inhibition compared to the controls

	Mean measured mecoprop-P concentration (mg a.s./L)	
	72h	96h
Area under the growth curve		
E_bC_{10}	24.5	36.0
E_bC_{20}	34.6	47.8
E_bC_{50}	57.8	77.3
LOEC	21.1	21.1
NOEC	10.3	10.3
Average specific growth rate		
E_rC_{10}	40.2	99.2
E_rC_{20}	60.9	119
E_rC_{50}	105	152
LOEC	41.8	86.5
NOEC	21.1	41.8

4. Algistatic/algicidal extension

Subcultures from the test cultures had re-established growth at the same rate as that of the control cultures after 5 days of incubation, indicating that at this concentration (200 mg a.s./L), mecoprop-P is algistatic.

5. Validity criteria

In accordance with OECD guideline no.201 (1984), the following conditions are considered to indicate a valid test:

- The cell concentration in the control cultures should have increased by a factor of at least 16 within three days; Control cell density increased from 1×10^4 to 40.3×10^4 cells/mL (40.3 x).
- Disappearance of the test substance from the water into the biomass does not necessarily invalidate the test: This was not confirmed. However 0 hour and 96 hour analytical measurements demonstrated stability of the test item within the test media.

Additionally, the modern guideline version (2006) requires the following in order to demonstrate a valid test:

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%: This cannot be confirmed from the study report.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. **For other less frequently tested species, the value should not exceed 10%:** This cannot be confirmed from the study report, but is 1.97% with regards to cell density.

III. CONCLUSIONS

The 72-hour E_rC_{50} (growth rate) and E_bC_{50} (biomass) values for mecoprop-P to *N. pelliculosa* were 105 and 57.8 mg/L, respectively, based on mean measured concentrations of mecoprop-P. The 72-hour NOEC values for growth rate and biomass were determined to be 21.1 and 10.3 mg/L, respectively.

RMS comments:

The study was adequately reported and conducted in accordance with the referenced guideline. Due to the age of the study additional validity criteria according to modern guideline versions were not monitored in the test and so cannot be confirmed from the report detail. The environmental conditions are judged to be satisfactory for organism performance, as the sole validity criteria relating to control cell density increase was met. It is noted that no supporting reference item toxicity data is provided in support of the study, so there is some uncertainty as to whether the test system was of adequate sensitivity. Overall the study is considered to be valid and acceptable for use in the risk assessment.

The agreed endpoints are as follows:

	Mean measured Mecoprop-P concentration (mg a.s./L)	
	72h	96h
Biomass		
E_bC_{10}	24.5	36.0
E_bC_{20}	34.6	47.8
E_bC_{50}	57.8	77.3
NOEC	10.3	10.3
Average specific growth rate		
E_rC_{10}	40.2	99.2
E_rC_{20}	60.9	119
E_rC_{50}	105	152
NOEC	21.1	41.8

All endpoints expressed in terms of mean measured concentrations of mecoprop-P.

Report:	CA 8.2.6.2/02, Burke, J. (2007)
Title	Mecoprop-P (DMA salt) algal growth inhibition assay <i>Skeletonema</i> Report No. ZZF0002/063525
Guidelines:	EEC C3 OECD 201
GLP:	Yes
Deviations	<p>In order to achieve suitable growth of <i>Skeletonema costatum</i> and maintain the test concentrations the following modifications to the test design were required:</p> <p>The replacement of the culture medium defined in ISO method 10253 with f/2 media as described by Guillard and Ryther 1963, Guillard 1975.</p> <p>It was considered necessary to prepare f/2 media without EDTA to prevent precipitation of the test substance.</p> <p>To prevent a build-up of algae around the meniscus line, the shaking speed of the incubator was increased to 130 RPM.</p> <p>To minimise the risk of cultures entering a lag phase during a 10-hour dark and 14 hour light cycle, it was necessary to conduct the test under continuous illumination.</p> <p>The cell density of the inoculum supplied by the Marine Biological Association was less than expected, therefore it was necessary to reduce the initial starting cell density of control and test cultures to 5.5×10^4 this deviates from the density (7.7×10^4 cells per ml) stated in the protocol.</p> <p><i>Skeletonema costatum</i> is a marine species and therefore additional monitoring of salinity was conducted. It was not stated in the protocol that these measurements would be conducted.</p>
Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test materials:** Mecoprop-P (DMA salt)
 - Description:** Clear brown liquid
 - Lot/Batch #:** 06/23
 - Purity:** 601.4 g Mecoprop-P/L
 - CAS #:** 66423-09-4
 - Stability of test compound:** Stable
 - Density:** 1.1337 g/mL
2. **Test animals**
 - Species:** *Skeletonema costatum*
 - Strain:** PLY/627

Source:	Plymouth Algal Culture Collection, Marine Biological Association of the United Kingdom
Culture medium:	Sterile f/2 diatom medium with exclusion of EDTA, and basal medium (natural filtered seawater)
Test vessel:	250 mL conical flasks closed with foam bung, 100 mL test media.
3. Environmental conditions	
Temperature:	21.8 – 24.5°C
pH:	7.7 – 9.4
Salinity:	36‰
Light intensity:	24-hr light at 4090 - 5020 Lux

B. STUDY DESIGN AND METHODS

1. Experimental dates:

20 April – 09 August 2006

2. Dose selection rationale

Two range finding studies were run at concentrations of 1, 10 and 100 mg a.s./L. The definitive test concentrations were based on the results of the range finding test and were nominal concentrations of 0, 3.15, 6.25, 12.5, 25, 50, 100 and 200 mg Mecoprop-P/L. Six control replicates were prepared for the control group, and 3 replicates per tested concentration.

3. Preparation of dosing mixtures

The test medium was prepared by adding the test substance (11.8, 23.6, 47.1, 94.3, 188.5 or 377 mg) to 1000 mL of algal culture medium f/2. An aliquot (3.5 mL) of the algal inoculum was added to each vessel (100mL) to give an initial cell density of 5.5×10^4 cells/mL.

4. Measurement of growth

Samples were taken from control and test flasks at 24, 48, 72 and 96 hours and the cell densities were measured using a haemocytometer. The estimate of diatom cell numbers in each sample was based on two counts, each comprising four or eight consecutive counts. The presence of any abnormal cells was also noted during counting.

5. Algistatic/algicidal extension

At the end of the definitive test, aliquots were taken from the highest concentration level, where growth had been severely inhibited and from the controls. A fresh, sterile culture medium was inoculated and the flasks were incubated for 5 days.

6. Statistics

Data were compiled in an Excel spreadsheet and analysed using the Curve Fit application of SAS 8.2 (SAS Institute 1999).

Non-linear regression was used to fit sigmoidal curves to the Area Under the Curve (AUC) and growth rate data.

95% confidence intervals for EC₅₀ were calculated using the likelihood ratio method (Donaldson and Schnabel, 1985). The EC₁₀ and EC₂₀ (with 95% confidence interval) were also estimated by reparameterising the formulae used to calculate the EC₅₀.

For AUC and growth rate, Williams' test (1971, 1972) was also used to compare each treated group with the control unless there was evidence of a non-monotonic dose response relationship, in which case Dunnett's test (1955, 1964) was used.

II. RESULTS AND DISCUSSION

1. Analytical results

The test concentrations were measured using mass spectrometry. At 0 hours duplicate samples were taken from each freshly prepared test media. At 96 hours replicate test vessel media was pooled per treatment and samples taken for analysis. 0 hour measured concentrations (as mecoprop-P) were in the range 94-100% of nominal concentrations and 96-hour old samples were in the range 92-102% of nominal. Results are conservatively based on mean measured concentrations of mecoprop-P.

2. Observations

After 96 hours of exposure, it was observed that diatoms exposed to nominally 200 mg a.s./L were swollen, elongated and some were irregular in shape. This duration of exposure is longer than the standard EU requirement of 72 hours.

3. Algal growth

Table B.9.2.6-07: Cell Densities and average growth rates after 72 and 96 hours

Nominal concentration as mecoprop-P (mg a.s./L)	Measured concentration as mecoprop-P (mg a.s./L)	Cell density (1 x 10 ⁵ cells/mL)		Inhibition of growth rate (%)	
		72-hr	96-hr	72-hr	96-hr
Control	0	14.8	24.2	--	--
3.15	3.09	12.5	23.2	5.0	1.2
6.25	6.19	13.1	20.0	3.7	5.3
12.5	12.2	14.4	21.6	0.7	3.3
25	23.3	14.2	27.6	1.2	-3.5
50	46.8	14.4	22.5	0.7	2.0
100	100	3.65	20.0	42.7	5.5
200	199	0.29	1.45	122.1	59.0

The E_bC₅₀ and E_yC₅₀ are presented in the table below. The results are expressed in terms of mean-measured levels of mecoprop-P. The 72h result is taken as the endpoint for the EU.

Table B.9.2.6-08: Percentage inhibition compared to the controls

	Mean measured mecoprop-P concentration (mg a.s./L)	
	72h	96h
Area under the growth curve		
E _b C ₁₀	63	67
E _b C ₂₀	70	76
E _b C ₅₀	84	95
LOEC	100	100
NOEC	47	47
Average specific growth rate		
E _r C ₁₀	86	124
E _r C ₂₀	92	149
E_rC₅₀	102	191
LOEC	100	199
NOEC	47	100

4. Algistatic/algicidal extension

Subcultures from the test cultures had re-established growth at the same rate as that of the control cultures after 5 days of incubation, indicating that at this concentration (200 mg a.s./L), mecoprop-P is algistatic.

5. Validity Criteria

In accordance with OECD guideline no.201 (1984), the following conditions are considered to indicate a valid test:

- The cell concentration in the control cultures should have increased by a factor of at least 16 within three days; Control cell density increased from 5.5×10^4 to 148×10^4 cells/mL (26.9x).
- Disappearance of the test substance from the water into the biomass does not necessarily invalidate the test; Samples taken at 96-hrs from additional replicates without algal cells did not show significantly increased test item recoveries to corresponding 'biotic' (i.e. with algal cells). Additionally 0 hr and 96 hour analytical measurements demonstrated stability of the test item within the test media.

Additionally, the modern guideline version (2006) requires the following in order to demonstrate a valid test:

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control must not exceed 35%; This cannot be confirmed from the study report.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. **For other less frequently tested species, the value should not exceed 10%;** This cannot be confirmed from the study report, but is 11% with regards to cell density.

III. CONCLUSIONS

The 72-hour E_rC_{50} (growth rate) and E_bC_{50} (biomass) values for mecoprop-P to *S. costatum* were 102 and 84 mg/L, respectively. The 72-hour NOEC values for growth rate and biomass were determined to be 47 and 47 mg/L, respectively.

RMS comments:

The study was adequately reported and mostly in line with the referenced OECD guideline, with associated validity criteria met. Some guideline deviations were apparent but these were mostly related to adjusting the test to suit the marine species of algae tested. It is noted that the additional validity criteria associated with the modern version of OECD guideline 201 were either not confirmed (due to a lack of replicate growth rate data in the report), or were narrowly exceeded (C of V for control replicate cell density was 11%). However, this was only narrowly exceeded and was based on surrogate parameter (2006 guideline refers to average growth rate). Overall control group performance is considered to be sufficient to demonstrate an acceptable test, based on the guideline followed at the time of the study. It is noted however, that no corresponding reference item data is reported; meaning that the sensitivity of the test system cannot be confirmed.

The agreed endpoints are as follows:

	Mean measured mecoprop-P concentration (mg a.s./L)	
	72h	96h
Biomass		
E_bC_{10}	63	67
E_bC_{20}	70	76
E_bC_{50}	84	95
NOEC	47	47
Average specific growth rate		
E_rC_{10}	86	124
E_rC_{20}	92	149
E_rC_{50}	102	191
NOEC	47	100

All endpoints expressed in terms of mean measured concentrations of mecoprop.

B.9.2.7. Effects on aquatic macrophytes*Reference*

Caley CY and Kelly CR (1999): Mecoprop-P dimethylamine salt. *Lemna* spp., growth inhibition test. Inveresk Research Lab., Report no. 17861. Mecoprop-p Task Force, BASF Doc ID 1999/1003117.

Fisher K 1999. Establishment of Methodology for the Analysis of Mecoprop-p Dimethylamine Salt in Aquatic Toxicology Media. Inveresk Report no. 17875. Mecoprop-p Task Force, BASF Doc ID 1999/1003116.

Previous evaluation:	Included in Addendum II to DAR (July 2002) for original a.s. approval
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Methods

The effect of mecoprop-P dimethylamine salt on the growth of *Lemna minor* was studied in a 7-day test according to the draft OECD guideline, 1998, and the US-EPA draft guideline 850.4400, 1996.

The test substance was mecoprop-P dimethylamine salt containing 765.7 g/l MCPP-P DMA salt with a specific gravity of 1.137 g/ml. Three vessels were prepared at each concentration (see the table below). Each vessel contained *Lemna* plants with a total of 15 fronds. In the semi-static test the test solutions were renewed on days 0, 3 and 5. The frond numbers were recorded on days 0, 3, 5 and 7 and the biomass dry weight was measured day 0 and at day 7. The average growth rate and the area under the curve were calculated. The pH of the test solutions was in the range 6.5-6.7 for fresh solutions and 8.2-10.2 at the end of renewal periods. Illumination took place by artificial daylight fluorescent tubes at light intensity 7870-9990 lux. The temperature was in the range 23-26°C.

The concentrations of MCPP-P (free acid) in the test samples were analysed and the corresponding concentrations of mecoprop-P dimethylamine salt were calculated. Because the mean measured concentrations were close to nominal, the nominal concentrations were used in the effect level calculations.

Table B.9.2.7-01: Nominal and measured concentrations (mg/l) in test solutions.

Nominal concentration									
MCPP-P DMA salt product	0	0.10	0.32	1.0	3.2	10	32	100	
equivalent MCPP-P DMA salt	0	0.067	0.22	0.67	2.2	6.7	22	67	
equivalent MCPP-P free acid	0	0.056	0.18	0.56	1.8	5.6	18	56	
Mean measured concentration									
MCPP-P free acid	0	0.052	0.16	0.49	1.7	5.5	17	55	
equivalent MCPP-P DMA salt	0	0.064	0.20	0.59	2.0	6.7	21	66	

Results

In all replicates at 67 and 22 mg MCPP-P DMA/l, distinct contortion of the fronds with chlorotic patches evident in some fronds and reduced root growth in new fronds were observed. At 6.7 and 2.2 mg/l similar effects were observed but these were less marked. At 0.67 mg/l some chlorotic patches were seen on new fronds and root growth appeared reduced compared to controls.

In control the mean frond number increased from 15 to 161 and the frond biomass increased from 4.86 mg to 18.04 mg during the 7 day test period (cf. test results below).

Table B.9.2.7-02: Summary of results.

	Days	Nominal concentration, mg MCP-P DMA salt							
		0	0.067	0.22	0.67	2.2	6.7	22	67
Mean frond numbers	0	15	15	15	15	15	15	15	15
	3	34.7	32.7	33	32.3	31.3	31.7	25.7	25.7
	5	74	73	64.7	62.3	62.7	62.7	42.3	39.7
	7	161	155	147.7	128	130	113.3	64.7	53
% inhibition	7		3.7%	8.3%	20.5%	19.3%	29.6%	59.8%	67.1%
Growth rate	0-7	0.339	0.333	0.327	0.306	0.308	0.287	0.208	0.18
% inhibition			1.8%	3.5%	9.7%	9.1%	15.3%	38.6%	46.9%
AUC	0-7	313.2	300.2	277	251	251.2	235.3	131	114
% inhibition			4.2%	11.6%	19.9%	19.8%	24.9%	58.2%	63.6%
Total biomass, mg	7	18.04	19.37	18.37	18.14	18.53	17.03	11.83	9.79
% inhibition*			-10.1%	-2.5%	-0.8%	-3.7%	7.7%	47.1%	62.6%

* Biomass minus the starting biomass in control and final treatment group.

The results on effect levels were based on the nominal concentrations of MCP-P DMA salt. The statistical analyses were performed according to OECD TG 221, draft 1998, for normalised Area under the curve (AUC) and growth rates for 0-7 days. The % inhibition were calculated for AUC, growth rate and final biomass relative to control and EC₅₀ estimated by probit analysis.

Table B.9.2.7-03: Effect results for Area under the curve (AUC), growth rate and final total biomass.

	Growth function	Day interval	EC ₅₀ (mg/l)	Confidence interval (mg/l)	LOEC (mg/l)	NOEC (mg/l)
MCP-P DMA salt	Growth rate	0-7	>67		0.67	0.22
	Area under curve	0-7	22.6	14.7-38.6	0.67	0.22
	Frond biomass	0-7	35.1		22	6.7
MCP-P	Growth rate	0-7	>56		0.56	0.18
	Area under curve	0-7	18.7	12.2-32.1	0.56	0.18
	Frond biomass	0-7	29.2		18	5.6

RMS Comments

The test is performed according to OECD TG221 (draft) with exception of pH. The pH in test solutions increased from 6.5-6.7 at beginning to 8.2-10.2 after 2-3 days at renewal. This is above the variation range of 1.5 pH units mentioned in the guideline. The increases in pH are observed at all levels including control and therefore not related to the test substance. The authors of the study suggest that the deviation could be related to increased plant growth, but ignores that pH only increased 1-1.5 pH unit in the range finding test. However, the result does not appear to be affected by this difference. The doubling time of frond number in the control $T_d = \ln 2 / \mu = 2.04$ days is below the 2.5 days required in the guideline as validation criteria. The pH change observed in the study is not considered to invalidate the test.

It is preferred to base estimates of toxicity on area under the curve rather than the average specific growth rate since the EC₅₀ could not be calculated from the average specific growth rate, i.e. EC₅₀ is 22.6 mg/l MCP-P DMA salt equal to 18.7 mg/l MCP-P.

It should be noted that LOEC is set at the lowest nominal concentration with and without significant effects, respectively, and not estimated from statistical extrapolation as recommended in the guideline.

RMS comments (renewal):

The notifier has not provided the report of this study for the purposes of active substance renewal. The validity of this study could not be confirmed by the RMS at renewal of the active substance. It is noted that a valid *Lemna spp* study (Hoberg and Witting, 1992) is available, and provides a lower endpoint for mecoprop-P than the reported endpoint for this study.

Reference

Hoberg, JR and Witting, R (1992): MCPP-P DMAS – Toxicity to the duckweed *Lemna gibba*. Springborn Laboratories, Inc. SLI Report # 92-3-4174.

Previous evaluation:	Included in Addendum III (December 2002) to DAR for original a.s. approval
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Methods

The effect of mecoprop-P dimethylamine salt on the growth of *Lemna gibba* strain G3 was studied in a 14-day semi-static test with 3-day renewal interval according to FIFRA guideline 122-2 and 122-3 (# 090591/FIFRA 122-2, 3).

The test substance was mecoprop-P dimethylamine salt containing 65.62 % as optically active salt (54.23 % as optically active acid) equivalent to 746.8 mg optically active DMA salt/mL.

Three replicate vessels were prepared at each test concentration based on a preliminary test. Test concentrations are giving in the table below. Each vessel contained 5 *Lemna* plants of 3 fronds each (a total of 15 fronds). The frond numbers were recorded on days 0, 3, 6, 9, 12 and 14.

The pH of the test solutions were in the range 4.9-5.7 throughout the exposure period and the light intensity was 3800-5400 lux. The temperature ranged from 24 to 26°C.

The concentrations of MCPP-P acid in the test samples were analysed and the corresponding concentrations of mecoprop-P dimethylamine salt were calculated. The mean measured concentrations were consistent between sampling intervals and averaged to 90 % of the control. The mean measured concentrations were used in the statistical analysis (William's Test) and in the effect level calculations.

The end point tested were the number of fronds recorded after 14 days exposure.

Table B.9.2.7-04: Nominal and measured concentrations (mg/l) in test solutions.

Nominal concentration						
MCPP-P DMA salt	0	0.64	1.3	2.5	5.0	10
Equivalent MCPP-P acid*	0	0.5	1.1	2.1	4.1	8.3
Mean measured concentration						
MCPP-P DMA salt	0	0.53	0.2	2.2	4.6	9.4
Equivalent MCPP-P acid*	0	0.44	0.17	1.8	3.8	7.8

*The equivalent concentrations of mecoprop-P acid are calculated by the RMS based on the molecular weight ratio 1.21 of the two compounds.

Results

In the controls the mean frond number increased from 15 to 430 during the 14-day test period (cf. test results below); all fronds in the controls appeared healthy except from 9 fronds day 9 which were observed to be slight sclerotic. Fronds exposed to the measured concentrations 1.2, 2.2, 4.6 and 9.4 mg a.s./L were chlorotic, small and curled with little root formation compared to control. Fronds exposed to the lowest measured concentration of 0.53 mg a.s./l were curled with less root formation than in the controls, and some chlorotic fronds were observed too.

Table B.9.2.7-05: Summary of results: Number of fronds at days and concentrations (in mg a.s. DMA salt/L), respectively.

Concentration mg/L	0	0.53	1.2	2.2	4.6	9.4
Day 0	15	15	15	15	15	15
Day 3	33	25	29	21	21	21
Day 6	84	63	73	41	40	31
Day 9	194	144	160	74	50	45
Day 12	311	261	251	96	55	41
Day 14	430	377	368	113	54	40

The results on effect levels were based on the measured concentrations of MCP-P DMA salt and are giving in the table below. The equivalent concentrations of mecoprop-P acid are calculated by the RMS based on the molecular weight ratio 1.21 of the two compounds.

Table B.9.2.7-06: Effect results for number of fronds after 14 days.

Test substance	EC50 (mg/l)	Confidence interval (mg/l)	LOEC (mg/l)	NOEC (mg/l)
MCP-P DMA salt	1.9	0.70 – 5.2	0.53	< 0.53
Equivalent MCP-P acid*	1.6	0.58 – 4.3	0.44	< 0.44

RMS Comments

The test was performed according to FIFRA guideline 122-2 and 122-3 and seems to be well conducted. NOEC is not estimated from statistical extrapolation but is set to be lower than the lowest test concentration.

The notifier argues that "Due to the wide range of CL values the results are very questionable and the study is considered invalid". There are no comments or discussion regarding the confidence limits in the study report. The RMS agrees that the confidence limits seems to be wide but does not find that this made the study invalid.

The present 14-days study on the duckweed *Lemna gibba* was conducted in 1992 (submitted December 2002 on request). A 7-day study on the duckweed species *Lemna minor* conducted in 1999 gave an EC50 value of 18.7 mg/l MCP-P acid with a confidence interval of 12.2 – 32.1 mg/l. Taking into account that the two studies are performed with two different species and different incubation periods the two results are not conflicting.

RMS comments (renewal):

The RMS has revisited the study report for the purposes of active substance renewal. In accordance with modern OECD guideline 221 the sole validity criterion was met (in the control group the doubling time of frond number was less than 2.5 days (60 h)). Environmental conditions were also appropriate for the species tested. However, it is noted that both the study guideline and the current active substance data requirements state that "*for Lemna species growth rate and yield, based on measurements of number of fronds and at least one additional measurement variable (dry weight, fresh weight or frond area).*" As such, although the study is valid, it does not fully address the data requirement and should be interpreted alongside other data on aquatic plants. The study endpoint is: **14-day EC₅₀ (frond number) = 1.9 mg/L (as DMA salt), equivalent to 1.6 mg/L as MCP-P**

B.9.2.8. Further testing on aquatic organisms

Ref.: IIA. 8.2.3. Ellgehausen, 1986: Accumulation and elimination of ^{14}C -mecoprop by Bluegill sunfish in a dynamic flow-through system.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The bioaccumulation of ^{14}C -mecoprop by Bluegill sunfish (*Lepomis macrochirus*) was studied in a 28 day dynamic flow-through system according to US-EPA guideline Subdivision E 71-6 (1982).

The test systems comprised three aerated tanks each containing 100 l of water. One tank served as control and two received mecoprop from a stock solution. The flow rate was 300 l/day. In each exposure tank, 150 fish were exposed to the nominal concentration of 1 mg/l ^{14}C -mecoprop, ring labelled 96.3% pure. The average fish weight were 2.4 g at day 0 and 3.0 g at day 28. The water temperature was 20°C.

Samples of 15 fish were taken at each sampling interval for analysis of residual radioactivity in non-edible part (head, viscera and fins), edible part (carcas, mainly filets) and the whole fish. After exposure was initiated samples were taken after 1 hour, and 3, 7, 14, 21 and 28 days. After 28 days of exposure, the water was exchanged by pure water (depuration phase) and samples were taken at day 1, 3, 7, 10 and 14. Samples of lyophilized and homogenized fish or fish parts were analysed by LSC and TLC.

Results

The bioconcentration factors (BCF) of 1.2, 5.5 and 3.0 were calculated in edibles, non-edibles and the whole fish, respectively, based on radioactivity (^{14}C -mecoprop equivalents).

The elimination half-lives of radioactivity from edibles, non-edibles and the whole fish were 7.8, 37.9 and 27.4 hours, respectively.

Analysis of the extractable radioactivity present in edible tissues at day 21 and 28 showed the presence of MCPP at an average amount of 0.24 mg/kg and two metabolites ranging 0.12 to 0.19 mg/kg and 0.05 to 0.13 mg/kg, respectively. A third metabolite was characterized as a methyl derivative of MCPP and ranged from 0.34 to 0.47 mg/kg.

The measured average concentration of ^{14}C -mecoprop in the water was 0.99 mg/l mecoprop equivalents.

Table B.9.2.8-01: Residues during exposure to 1 mg/l mecoprop

Part of fish	Residues in mg MCPP equivalents/ kg fish, fresh weight after:						
	C*	1 h	3 d	7 d	14 d	21 d	28 d
Edible	0.39	0.39	0.74	0.93	0.99	1.19	1.35
Non-edible	0.33	0.73	4.20	4.33	5.33	5.62	5.35
Whole fish	0.37	0.56	2.15	2.35	2.91	2.98	3.09

C*: Control fish. Background values, average of day 0 and 28 measurements. h: hour. d: days.

Table B.9.2.8-02: Residues during depuration. Day 0 of depuration = day 28 of exposure

Part of fish	Residues in mg MCPPE equivalents/ kg fish, fresh weight after					
	1 h	3 d	7 d	14 d	21 d	28 d
Edible	1.35	0.45	0.19	0.07	0.05	0.03
Non-edible	5.35	2.20	1.09	0.87	0.65	0.64
Whole fish	3.09	1.21	0.57	0.40	0.31	0.29

RMS Comments

The study is acceptable. mecoprop has a low bioaccumulation potential and an elimination half-life of about 1 day for the whole fish.

RMS comments (renewal):

The RMS has revisited the study report for the purposes of active substance renewal. Study confirmed as GLP. Validity criteria (as per OECD 305 – 1012 version) were met as follows:

The water temperature variation is less than $\pm 2^{\circ}\text{C}$ = Maximum range within a single vessel = 1.0°C

- The concentration of dissolved oxygen does not fall below 60% saturation = Minimum recorded = $7.3 \text{ mg/L} \approx 80\% \text{ ASV at } 20^{\circ}\text{C}$.
- The concentration of the test substance in the chambers is maintained within $\pm 20\%$ of the mean of the measured values during the uptake phase = achieved. Reported concentration range in treated tanks = $0.99 \pm 0.05 \text{ mg/L}$, versus a nominal concentration of 1.0 mg/L
- The concentration of the test substance is below its limit of solubility in water, taking into account the effect that the test water may have on effective solubility = as nominal concentrations were almost exactly met and no observations of undissolved test material were reported it is assumed that the test item was fully soluble in the test system at the treated concentration.
- The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical = the report states that in a pre-test at 0, 1 and 10 mg/L no mortality was seen in the test organism. During the study there were no reported fish mortalities.

As such the RMS concludes the study as suitable to provide a bioconcentration factor for the test item in fish. The agreed endpoint is a whole fish $\text{BCF} = 3.0$. It should be noted that a fish bioconcentration study is not triggered since the $\log \text{Pow}$ for mecoprop-P is less than 3.

Report:	CA 8.2.8/01, Simmons, K. (2015)
Title	Position Paper: Assessment of the presence or absence of the parent toxophore in o-cresol (metabolite of Mecoprop-P) Nufarm UK Ltd. Report No. Wyke_2015_043
Guidelines:	N/A
GLP:	No

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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Methods

An assessment of the potential toxicity of the aqueous photometabolite o-cresol was made by considering the presence or absence of the parental toxophore (from mecoprop-P), assessing available data and analysing QSAR results.

Two relevant toxophores were identified within the parent compound mecoprop-P, namely arylalkanoic acid and benzoic acid. When comparing these toxophores with the structure of the metabolite o-cresol it was clear that the toxophores of mecoprop-P had been lost in o-cresol.

Table B.9.2.8-03: Identified toxophores in the active substance mecoprop-P

Benzoic acid

Table B.9.2.8-04: Structure of photometabolite o-cresol and structural features

Name
o-cresol

Although Nufarm do not hold data on the metabolite o-cresol there is information obtainable on the ecotoxicology of o-cresol available on the ECHA (European CHemicals Agency) website, via REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) registration of the chemical. The available endpoints from the ECHA data set are shown in the below table:

Table B.9.2.8-05: Available aquatic toxicity data with metabolite O-cresol (Source: ECHA website)

Aquatic species	Endpoint type	Toxicity of O-cresol (mg/L)
<i>Salmo trutta</i>	Fish 96-hr acute LC ₅₀	6.2
<i>Oncorhynchus mykiss</i>	Fish 96-hr acute LC ₅₀	7.0
<i>Salvelinus fontinalis</i>	Fish 96-hr acute LC ₅₀	7.2
<i>Daphnia magna</i>	Aquatic invertebrate 48-hr acute EC ₅₀	15.7
<i>Daphnia pulax</i>	Aquatic invertebrate 48-hr acute EC ₅₀	9.6
<i>Daphnia cucullata</i>	Aquatic invertebrate 48-hr acute EC ₅₀	16.4
<i>Selenastrum spp.</i>	Algal 96-hr EC ₅₀	100

A QSAR assessment was also undertaken. Upon assessing QSAR results it was determined that the most sensitive aquatic species will be Daphnids. The QSAR modelled data is provided in the below table:

Table B.9.2.8-06: QSAR generated aquatic toxicity data with metabolite O-cresol

Aquatic organism group	Endpoint type	Toxicity of O-cresol (mg/L)
Fish acute	96-hr LC ₅₀	16.9
Fish chronic	Not given	1.7
Daphnid acute	48-hr EC ₅₀	5.2
Daphnid chronic	Not given	1.0
Green algae	96-hr EC ₅₀	23.9
Aquatic plant <i>Lemna</i>	7-day EC ₅₀	11.9

Although the most sensitive species predicted in the model does not match that of the actual data, where the critical species was the fish, the actual predicted RAC is very similar to that shown by the data set available on the ECHA website.

III. CONCLUSIONS

Taking into consideration all available information it can be concluded that the aqueous photolysis metabolite o-cresol has lost the toxophore present in the parent substance mecoprop-P. Available data and QSAR predictions also confirm that o-cresol is expected to be less toxic than mecoprop-P. For these reasons, in line with EFSA (2013), it is considered appropriate to use the critical endpoint for the parent mecoprop-P, in place of generating data on the metabolite o-cresol.

RMS comments:

The RMS agrees on the basis of the argumentation provided by the applicant that the toxophore responsible for the herbicidal activity of the active substance is not present in the metabolite o-cresol. The above position paper has been considered by the RMS and considered to be reliable in its sourcing and generation of data. As such it is proposed by the RMS that the lowest endpoint from the above datasets per organism group be used in the aquatic risk assessment in order to provide quantitative assessment of the potential risk from the metabolite in the aquatic environment. The critical endpoints for use in the risk assessment are therefore as follows:

Aquatic organism group + species (if provided)	Endpoint type	Toxicity of O-cresol (mg/L)
Fish acute (<i>S.trutti</i>)	96-hr LC ₅₀	6.2 ¹
Fish chronic	Not given assumed NOEC	1.7 ²
Daphnid acute	48-hr EC ₅₀	5.2 ²
Daphnid chronic	Not given assumed NOEC	1.0 ²
Green algae	96-hr EC ₅₀	23.9 ²
Aquatic plant <i>Lemna</i>	7-day EC ₅₀	11.9 ²

¹ study data via REACH database

² QSAR-predicted toxicity

It is noted that no data, study generated or modelled is available with the aquatic plant group *Myriophyllum spp.* With regards to the active substance this group was the most sensitive, more so than the other aquatic plant species test; *Lemna gibba*, by about a factor of 1000. This is considered further in the risk assessment for aquatic organisms (Volume 3 (CP) B.9.4).

B.9.3. EFFECTS ON ARTHROPODS**B.9.3.1. Effects on bees*****B.9.3.1.1. Acute Toxicity to Bees***

Ref.: IIA. 8.3.1/01. Hoxter & Lynn, 1991: MCPP-P DMAS: An acute contact toxicity study with the honey bee.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The acute toxicity of MCPP-P DMA salt to honey bees (*Apis mellifera*) was studied in a contact test according to US-EPA FIFRA guideline 141-1.

MCPP-P DMA salt containing 65.62% as optical active salt (54.23% as acid equivalents) and 68.4% as total salt was prepared in five nominal treatment levels representing 1.6, 3.1, 6.3, 12.5 and 25 µg/bee (based on the whole product) along with solvent control (acetone) and negative control. Two replicates were tested at each dose with 25 bees per replicate. The bees were 1-6 days old when they were exposed by applying 2 µl of the test solution on the thorax and/or abdomen and observed for 48 hours.

The doses and LD₅₀ value reported were based on the whole product.

The toxicity was classified according to Atkinson:

Highly toxic:	LD ₅₀ < 2 µg/bee
Moderately toxic:	LD ₅₀ ≥ 2 µg/bee but < 11 µg/bee
Relatively non-toxic:	LD ₅₀ ≥ 11 µg/bee

Results

Contact LD₅₀ (48h) was determined to be > 25 µg/bee, i.e. MCPP-P DMA was classified as relatively non-toxic to bees. NOEC based on the study was 25 µg/bee.

Table B.9.3.1-01: Accumulated mortality

Time	Accumulated mortality in % (of 50 bees) after exposure to (µg/bee)						
	0	SC	1.6	3.1	6.3	12.5	25
Day 0	0	4	0	2	0	0	0
Day 1	4	6	0	2	0	4	6
day 2	6	6	0	2	2	4	8

SC: solvent control (2 µl acetone/bee)

RMS Comments

The study was acceptable. The test substance was identified as batch 9358/7 probe 44.

The contact test in which the bees are exposed by droplet application simulates a likely exposure route to bees and other non-target insects. The exposure may occur through absorption or from grooming.

RMS comments (renewal):

The RMS has revisited the study report for the purposes of active substance renewal. Much of the study methodology is out-dated compared to current (OECD) guidance. It is further noted that not associated toxic reference data was provided. As such the sensitivity of the test organism cannot be confirmed. As this is a validity criterion under modern guidance the conclusion of the RMS is that the study cannot be used to provide a reliable endpoint for acute contact toxicity.

Reference

Weyman GS (1999): MCCP-P DMA. Acute contact and oral toxicity to honeybees. Covance, UK, Report No. 1149/24-D2145 for the MCPP Task Force Final Report 99/10137.

Previous evaluation:	Included in Addendum II to DAR (July 2002) for original a.s. approval
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Methods

The acute contact and oral toxicity of formulated MCPP-P DMA to honeybees was determined in a 48 hour laboratory test performed in accordance with the EPPO guideline no. 170, 1992.

The test animal was honeybees *Apis mellifera* exposed in cylindrical test cages 170 mm long and 45 mm in diameter. Each cage contained 10 adult worker honeybees. Three replicates were used for each exposure level. The test substance was mecoprop-P dimethylamine salt (MCPP-P DMA) formulated as water soluble concentrate liquid (SL) containing 765.7 g MCPP-P DMA/l. The exposure levels were control, 6.25, 12.5, 25, 50 and 100 µg MCPP-P/bee in both contact and oral exposure routes. Contact toxicity test were made with test substance diluted in water and added surfactant. The doses were applied by pipette to anaesthetised bees by placing a 1 µl droplet onto the dorsal thorax of each bee. In the oral toxicity test, the dose was given as test substance diluted in 0.2 ml sucrose solution as food to starved bees. After consumption of the dose, it was replaced with normal food as sucrose solutions. A reference substance, dimethoate, was included in the test. The temperature was 24°C and the humidity varied between 54 to 81% relative humidity. Assessments of mortalities and sub-lethal effects were made after 1, 2, 4, 24 and 48 hours. For the toxicity data a probit analysis was performed to derive LD₅₀.

Results

In the contact toxicity test, single mortalities were observed in the exposed groups but were not considered treatment related (please see the table below). No significant sub lethal effects were observed in the test at 24 and 48 hours. In the oral toxicity test no mortalities were observed up to and including 50 µg MCPP-P DMA/bee.

The contact and oral LD₅₀ (48 h) was observed to be >100 µg MCPP-P DMA/bee (131 µg formulation/bee) which was the highest dose tested.

The NOEL contact (48 h) was ≥100 µg MCPP-P DMA/bee and the NOEL oral (48 h) was 50 µg MCPP-P DMA/bee (65 µg formulation/bee).

Table B.9.3.1-02: Mortality: Number and (%).

Exposure route	n	Exposure concentration, nominal µg a.s./bee					
		0	6.25	12.5	25	50	100
Contact, 48 h	30	2 (7%)	0	1 (3%)	0	1 (3%)	1 (3%)
Oral, 48 h	30	0	0	0	0	0	12 (40%)

RMS Comments

The response to the reference substance was in agreement with previous studies and indicated that the honeybees responded normally to the test system. The study was performed in accordance to the EPPO guideline.

The effect values was not stated for MCPP-P free acid, but based on the molecular ratio between MCPP-P DMA and MCPP-P (259.72/214.65), RMS has calculated the values, see the table below.

Table B.9.3.1-03: Toxicity of mecoprop-P to honey bee.

Bee toxicity	ED ₅₀ (48 h)	NOEC (48 h)
	µg/bee	µg/bee
Contact	>83	>83
Oral	>83	41

RMS comments (renewal):

The RMS has revisited the study report for the purposes of active substance renewal. The study appears to have been conducted in good adherence with the guideline referenced, which is largely similar to modern OECD guidelines 213 and 214. Control mortality in each test did not exceed 10%, and the 24-hour LD₅₀ for the toxic reference item in the oral test was within the range specified for a valid test according to OECD 213 (0.133 µg a.s./bee, range = 0.1-0.35 µg a.s./bee). However, the contact route toxic reference LD₅₀ was in excess of the modern validity criterion range (0.558 µg a.s./bee versus a required range of 0.1 – 0.35 µg a.s./bee). It is noted that the report states that this was “consistent with values determined previously at the test facility”. Given this fact, and the point that the study was otherwise well conducted the RMS judges that the contact endpoint from the study is still suitable for use.

The agreed endpoints are as follows:

Bee toxicity route	LD ₅₀ (48 h) as MCPP-P DMA salt	LD ₅₀ (48 h) as MCPP-P (acid)
	µg/bee	µg/bee
Contact	>100	>83
Oral	>100	>83

B.9.3.1.2. Effects on honeybee development and other honeybee life stages

Report:	CA 8.3.1.3/01, Kleebaum, K. (2014)
Title	Kleebaum, K. (2014) Acute toxicity of mecoprop-P technical acid to honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (<i>in vitro</i>) BioChem agrar Report No. 14 10 48 023 B Date: 08 December 2014
Guidelines:	OECD 237 Guidelines for testing chemical “Honey bee (<i>Apis mellifera</i>) larval toxicity test, single exposure” (2013)
GLP:	Yes
Deviations	None

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS**A MATERIALS**

- 1. Test Material:** Mecoprop-P technical acid
Batch no.: 3860

Analysed purity:	92.63%
Description:	Light yellow solid
2. Toxic reference:	Dimethoate, 99.8% purity (w/w)
3. Test Organism	
Species:	Honeybee (<i>Apis mellifera carnica</i> P.)
Age:	1 st instar larvae 1 day old
Source:	3 x healthy, queen-right colonies (each replicate per tested group contained larvae from a single colony); Bienenfarm Kern GmbH, Leipzig, Germany
Acclimation:	Honeybee larvae were acclimated to the test cells for 3 days prior to date of application of the test item.
Feed:	An aqueous sugar solution was prepared freshly and mixed with royal jelly every day prior to feeding. The volumes and contents of the different diets for different time points are shown in Table CA B.9.3.1-04.

Table B.9.3.1-04: Feeding scheme

Test day	1*	2	3	4**	5	6	7***
Volume of diet per larva [uL]	20	-	20	30	40	50	-
Composition of diets, based on (w/w):		-					-
Royal Jelly	50%		50%	50%			
Sugar Solution	50%		50%	50%			
Composition of sugar solution, based on (w/v):		-					-
Glucose	12%		15%	18%			
Fructose	12%		15%	18%			
Yeast	2%		3%	4%			

* Day of grafting

** Day of application

*** Day of final assessment

Housing:	Test cages were crystal polystyrene grafting cells (CNA Nicoplast, internal diameter 9mm). Grafting cells were placed in 48 well plates, which were filled up to 1/3 with a piece of wetted dental roll. Larvae were individually housed in the cells, with 12 larvae from a single colony comprising 1 replicate. 3 replicates per group were prepared and housed on a single plate.
4. Environmental Conditions:	Climatic chamber (Binder KBF 720) Temperature: 34.0 – 35.0°C Relative humidity: 93-96% Illumination: Constant darkness Ventilation: Via air-conditioning equipment of the climate chamber

B. STUDY DESIGN AND METHODS

1. In-life dates:	13 June 2014 – 19 June 2014
2. Test system	
Duration of study:	7 days total, with 3 days (72 hours) exposure to mecoprop-P within the diet.
Guideline deviations reported by study director:	None reported.
Test concentrations:	99.2, 49.6, 24.8, 12.4, and 6.2 µg mecoprop-P/larva (corresponding to 2.925, 1.463, 0.731, 0.366, 0.183 g mecoprop-P/kg food)
Parameters measured:	Mortality – Number of dead larvae (immobile larvae or ones which did not react to a contact stimulus were noted as dead) were recorded daily (24, 48 and 72 h after application).
	Other observations – E.g. morphological differences compared to the control (72 h after application).
	Presence of unconsumed food – Qualitatively described at 72 h after application.
Analytical verification:	Stock solution A (used to dose highest dose group) was sampled in duplicate. Determination of the active ingredient content within the sugar solution was conducted using HPLC with UV-detection.
3. Test preparations:	<p>Pre-treatment culturing conditions:</p> <p>Bee colonies producing the larvae were held under field conditions in hives including a healthy queen and brood in egg, larval and pupal stages.</p> <p>Method of producing L1 larvae:</p> <p>On day -3 the queen of each colony was confined in an empty brood comb, where the queen laid her eggs solely in this comb. The caging time for this was approximately 30 hours. In the afternoon of day -2 the queen was released. The comb was then checked for presence of eggs and placed near to frames containing open brood in the hive. The eggs were incubated in the hive between day -2 and day 1.</p> <p>Grafting:</p> <p>On day 1 the combs containing larvae were transported from the hives to an acclimatized laboratory room. Larvae were transferred from the combs to the cells using a suitable grafting tool. Each of the three replicates represents larvae originating from a different colony to exclude colony effects.</p>
4. Methodology:	<p>All larvae were checked prior to application, with all sick or dead larvae being exchanged for normally developed individuals.</p> <p>Three replicates of the control, test item dosed and reference item dosed were used, with 12 larvae per replicate.</p> <p>The test item was mixed into sterile filtered aqueous sugar solution (Stock A). Dilutions were then prepared from Stock A by adding further sugar solution. In order to dissolve the test item acetone was used as a solvent carrier. To each stock solution royal jelly was added at a ratio of 1:1, based on w/w, to reach the final test concentrations.</p> <p>All sugar solutions were warmed to 35°C in a water bath prior to feeding. Each larva was fed separately with a sterile pipette. The food</p>

drop was placed next to the larvae to avoid drowning.

After consumption of the test item on day 4, larvae of all treatment groups were fed with untreated diet for the remaining days.

5. Statistical analysis:

For statistical calculation of the mortality results the Fisher's Exact Binomial test (with Bonferroni correction) was used, with a significance level of $p \leq 0.05$ (one-sided greater).

All statistical calculations were performed in ToxRat Professional 2.10.06 (2010).

II. RESULTS

Validity criteria:

- Larvae mortality in the control must be $\leq 15\%$ across all control replicates = 0% / 5.6% (control / solvent control)
- Toxic reference item mortality must be $\geq 50\%$ at 8.8 ± 0.5 $\mu\text{g/larvae}$ = 55.6% at 8.8 $\mu\text{g/larvae}$
- Analysed concentration of active substance in a sample must be $\pm 20\%$ of the nominal concentration = 101% nominal (stock solution)

Mortality:

After 72 hours oral exposure a mortality of 0.0% was observed in the solvent-free control, while in the acetone solvent control a mortality of 5.6% occurred. In the test item group, uncorrected mortalities ranged 0.0 to 61.1%. Statistically significant mortality occurred at the highest test item dose of 99.2 μg mecoprop-P/larva. Mortality in the dimethoate toxic reference was 55.6% (uncorrected). Table B.9.3.1-05 displays the mortality results in more detail.

Table B.9.3.1-05: Mortality results

Treatment Group	Dosage [μg mecoprop-P/larvae]	Concentration [g mecoprop-P/kg food]	Cumulative mortality after 72 hours (mean) [%]	
			Absolute	Corrected
Control	-	-	0.0	-
Solvent Control	-	-	5.6	-
Test item	99.2	2.925	61.1*	58.8
	49.6	1.463	5.6	0.0
	24.8	0.731	0.0	0.0
	12.4	0.366	2.8	0.0
	6.2	0.183	5.6	0.0
Reference item	8.8	0.260	55.6	55.6

*Statistically significant difference in pairwise comparison between treatment and untreated control.

Observations:

Smaller body size of surviving larvae and/or leftover food on D7 occurred at an increased rate (52.4 % and 16.7 % at the two highest test item groups (99.2 and 49.6 µg a.s./larva). In the lower test item groups the rate of remaining food/smaller body size was below or at the level of the solvent control. Table B.9.3.1-06 displays the observations in more detail.

Table B.9.3.1-06: Observations

Treatment Group	Dosage [µg mecoprop-P/larvae]	Concentration [g mecoprop-P/kg food]	Observations [#] [%]
Control	-	-	0.0
Solvent Control	-	-	3.0
Test item	99.2	2.925	52.4
	49.6	1.463	16.7
	24.8	0.731	0.0
	12.4	0.366	2.8
	6.2	0.183	3.0
Reference item	8.8	0.260	22.2

[#]Observations include large quantities of remaining food and/or smaller body size of larva.

Analytical work:

The concentration of mecoprop-P in an analysed sample of the test item stock solution (nominally 2.925 g a.s./kg food) had a 101% recovery rate, thus confirming the dosing of the test item.

III. CONCLUSIONS

In the test item group uncorrected mortalities ranged 0.0 to 61.1% after 72 hours, with the highest dose of 99.2 µg mecoprop-P/larva showing statistically significant mortality when compared with the control. Smaller body size and/or remaining food occurred at an increased rate (52.4 % and 16.7 % at the two highest test item groups (99.2 and 49.6 µg a.s./larva). In the lower test item groups the rate of remaining food/smaller body size was below or at the level of the solvent control.

In an acute toxicity study with honey bee larvae dosed via the diet with mecoprop-P technical acid the endpoints were determined to be:

Table B.9.3.1-06: Study endpoints

Treatment	Test item dose (µg a.s./larva)				Test item concentration (g a.s./kg food)			
Endpoint type	NOED	LD ₁₀	LD ₂₀	LD ₅₀	NOEC	LC ₁₀	LC ₂₀	LC ₅₀
Endpoint	49.6	43.7	55.7	89.4	1.463	1.290	1.641	2.636

RMS comment:

The study was well reported and conducted in close adherence with the relevant OECD test guideline. All associated validity criteria were met and there were no notable deviations. The study is considered valid and acceptable for risk assessment purposes. The agreed endpoints are as follows:

Treatment	Test item dose (µg a.s./larva)			Test item concentration (g a.s./kg food)		
Endpoint type	NOED	LD ₁₀	LD ₅₀	NOEC	LC ₁₀	LC ₅₀
Endpoint	49.6	43.7	89.4	1.463	1.290	2.636

B.9.3.1.3. Sub-lethal effects to bees

Report:	CA 8.3.1.4/01, Mack, P. (2012)
Title	LAF-74: A semi-field study to investigate residues in honeybee products and honeybee larvae (<i>Apis mellifera carnica</i> L.; Hymenoptera, Apidae) in <i>Phacelia tanacetifolia</i> in Germany in 2011 Report No. S11-02084
Guidelines:	IVA (Beutel <i>et al.</i> , 1992) OEPP/EPPO (2010)
GLP:	Yes (excepting local weather data)
Deviations	None

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS**A. MATERIALS**

1. **Test materials:** LAF-74
 - Description:** Amber to brown liquid
 - Lot/Batch #:** 15
 - Purity:** 603 g 2,4-D/L
 - Product Density:** 1.2024 g/mL
 - CAS #:** 2008-39-1
 - Stability of test compound:** Stable
2. **Application:** Calibrated portable boom sprayer at 750 g a.s./ha (in 400 L/ha volume)
3. **Test animals**
 - Species:** Honey bee (*Apis mellifera carnica* L.)
 - Source:** Not stated; confirmed as healthy colonies with 1 x egg-laying queen; 20 combs/hive; ≥ 4 brood combs/hive, 6-11 honey and pollen combs/hive.
 - Acclimatisation period:** 4 days
 - Diet:** No artificial food was offered; foraging on target crop.
 - Housing:** Free standing hives in tunnels under natural field conditions

B. STUDY DESIGN AND METHODS**1. In life dates:**

28 July – 18 October 2011

2. Animal assignment and treatment

Four colonies (hives) of the honeybee, *Apis mellifera* L. (Hymenoptera, Apidae) and brood comprising at least 4 brood combs with all brood stages and 6 - 11 honey and pollen combs were used. For the test, healthy colonies with young honeybees, one egg-laying queen and with two bodies (lower body = brood chamber; upper body = honey comb box) including 20 combs were used.

Two bee colonies were introduced per tunnel 4 days before the application during bee-flight. Each tunnel was 250 m² (50 x 5 m) and was enclosed with a 1.5mm mesh gauze. A 60 cm wide path was marked along the central length of each tunnel by removal of the crop plant. A container filled with water was placed into each tunnel as water supply for the bees. The surface of the water was covered with cork to prevent the bees from drowning. During the application the water containers were taken out of the tunnels and the hives were covered with a plastic foil to avoid direct spray contamination.

Plots contained blooming *Phacelia tanacetifolia* at BBCH 62. Application was conducted during flowering and during daily bee-flight using a calibrated portable boom sprayer that simulates a commercial application. At application ≥ 20 foraging bees/m² were observed, confirming bee activity.

3. Dose selection

The study included one treatment group, at 750 g a.s./ha in 400L water/ha and comprised two tunnel replicates of approximately 250 m². Pre-application sampling was considered to provide control residue levels in the various organism compartments (nectar, pollen, larvae).

4. Observations

Table B.9.3.1-07: Study conduction timetable

3 rd sampling
2 nd sampling
4 th sampling
5 th sampling
3 rd sampling
DAA: days

Sampling of worker bees for stomach nectar

On each sampling day, one sample of at least 300 forager bees was taken per hive. After the sampling each sample was divided into two sub-samples, each sub-sample with at least 150 bees, one for

preparation and one as a retained sample (both stored at $\leq -18^{\circ}\text{C}$). The honey stomach contents were sampled and pooled. To sample the nectar/honey from bee stomach contents the sampled bees were thawed, and the honey stomachs were dissected and weighed as a sample total.

Sampling of foraged pollen

The pollen retrieved by the bees was collected in front of the colonies on five sampling days. As there was not enough pollen at the control sampling (2DBA), the procedure was repeated two times (1DBA and 0DBA). For the residue analysis the pollen from the day of application was used (0DBA). The timing for the samplings was 2 to 0 DBA, 1, 2, 3 and 5 DAA. Pollen traps were fixed on the hive in each treatment group during the experimental phase in the tunnels. The pollen traps were emptied seven times during the experimental phase with a sampling period of 2-10 hours. In between sampling dates the pollen traps were left open to ensure the healthy condition of the bee hive. If possible the collected amount of pollen was divided into two sub-samples containing approximately the same amount of pollen. All samples were stored at $\leq -18^{\circ}\text{C}$ until analysis.

Sampling of larvae

Honeybee larvae were collected inside the tunnels on three sampling dates during the experimental period. The timing for the samplings was 2 DBA, 3 and 5 DAA. On each sampling day 60 larvae were taken from the brood combs of each hive, uncapped honeybee larvae (stage 5-9 days) were sampled out of the individual wax cells and placed into small plastic containers. The sampling took place after the forager bee sampling and pollen sampling to keep disturbance to a minimum. All samples were stored at $\leq -18^{\circ}\text{C}$ until analysis.

Weather conditions during the study

Air temperature, relative humidity and daily rainfall were monitored at the laboratory weather station located approximately 4 km from the test site. During application of the test item and observation events the temperature, humidity, cloud cover and wind speed were measured at the test site.

5. Analysis

2,4-D was extracted by means of cold extraction with acetonitrile/water (1:1, v/v). Parent compound concentrations were determined using HPLC with MS/MS detection.

II. RESULTS AND DISCUSSION

A. BEE BROOD

The colony strength before set-up in the tunnels ranged from 7063 to 15063 honeybees. All colonies used for the study had brood of all stages (eggs, larvae, sealed brood). Food (nectar and pollen) was also present in all colonies with a higher percentage of nectar (15.5 to 30.3 %) compared to pollen (3.1 to 6.3 %).

B. ANALYTICAL RESULTS

No residues of 2,4-D at or above the respective limit of detection (LOD) levels (0.003 mg/kg for nectar, pollen and honeybee larvae) were found in any of the untreated samples that were taken from 2 days (nectar and larvae) and from the day of application (pollen) before application (2 and 0 DBA) serving as control in this study.

In nectar samples from forager bees, residues of 2,4-D were determined in all samples for all sampling dates during bee exposure. One day after application (1DAA) mean residues of 2,4-D were 2.45 mg/kg and declined to 0.31 mg/kg and 0.41 mg/kg two days and three days after application (2DAA and 3DAA), respectively. Five days after application (5DAA) the mean residue of 2,4-D was 0.07 mg/kg.

In pollen samples from pollen traps residues of 2,4-D were determined for all samplings during bee exposure. Mean residues of 2,4-D were 75.3 mg/kg one day after application (1DAA) and declined to 13.9 mg/kg two days after application (2DAA). Three days after application (3DAA) residues of 2,4-D declined further to 3.13 mg/kg. Five days after application (5DAA) residues were similar to those 3DAA at 7.67 mg/kg.

Table B.9.3.1-08: Residues of 2,4-D in nectar and pollen of flowering *Phacelia tanacetifolia* following application of 750 g a.s./ha

Timing	Treatment	Residue in nectar (mg/kg)	Mean residue in nectar (mg/kg)	Residue in pollen (mg/kg)	Mean residue in pollen (mg/kg)
2DBA	Control (untreated)	n.d.	n.d.	n.d.	n.d.
		n.d.		n.d.	
		n.a.		n.d.	
		n.d.		n.d.	
1DAA	750 g a.s./ha	1.81	2.45	68.2	75.3
		0.68		66.2	
		6.40		81.9	
		0.91		84.8	
2DAA	750 g a.s./ha	0.49	0.31	12.6	13.9
		0.26		14.0	
		0.18		13.4	
		0.31		15.6	
3DAA	750 g a.s./ha	0.09	0.41	2.74	3.13
		0.84		2.73	
		0.43		3.21	
		0.28		3.85	
5DAA	750 g a.s./ha	0.06	0.07	7.28 / 7.65 *	7.67 ¹
		0.12		6.99 / 7.04 *	
		0.03		6.86 / 8.97 *	
		0.05		8.22 / 8.38 *	

DBA days before application during bee flight

DAA days after application during bee flight

n.d. not detected (residue value was less than the LOD of 0.003 mg/kg)

n.a. not available (sample destroyed during analysis)

* second (retained) sample

¹ mean residues of primary and secondary (retained) sample

In honeybee larvae samples, residues of 2,4-D were determined for two samplings during bee exposure. Three days after application (3DAA) mean residues were 2.41 mg/kg and declined to 0.98 mg/kg five days after application (5DAA).

Table B.9.3.1-09: Residues of 2,4-D honey bee larvae following application of 750 g a.s./ha to flowering *Phacelia tanacetifolia*

Timing	Treatment	Residue in nectar (mg/kg)	Mean residue in larvae (mg/kg)
2DBA	Control (untreated)	n.d.	n.d.
		n.d.	

Timing	Treatment	Residue in nectar (mg/kg)	Mean residue in larvae (mg/kg)
		n.d.	
		n.d.	
3DAA	750 g a.s./ha	2.01	2.41
		5.58	
		0.58	
		1.46	
5DAA	750 g a.s./ha	0.76	0.98
		2.20	
		0.31	
		0.64	

DBA days before application during bee flight

DAA days after application during bee flight

C untreated control samples taken before application

T test item group

n.d. not detected (residue value was less than the LOD of 0.003 mg/kg)

C. WEATHER CONDITIONS

Local weather: During the pre-exposure phase (-4 to -1 DAA) total rainfall was 2.9 mm, air temperature was in the range 11.7 – 24.0 °C and mean daily humidity was 65.4 – 70.1%. During the exposure phase (0DAA to 5DAA) rainfall (sum of 27.3 mm), relative mean daily air humidity from 54.4 to 74.8 % and minimum and maximum temperatures of 10.1 and 27.8 °C were recorded.

Tunnel conditions at application (0 DAA): Temperature in the tunnels was recorded as 29.6 – 30.5 °C. Cloud cover was observed as 10% and the target was noted as dry. Humidity was in the range 40.2 – 48.3% and wind speed was 0.2-0.3 m/s.

III. CONCLUSIONS

Residues of 2,4-D were determined in nectar samples of all hives, in which the test item was sprayed during flowering and daily bee-flight. Mean residues were 2.45 mg/kg one day after the application (1DAA), declining to 0.07 mg/kg at the last sampling, five days after application (5DAA).

In pollen samples residues of 2,4-D were determined for all hives during bee exposure. Mean residues were 75.3 mg/kg one day after application (1DAA), declining to 3.13 mg/kg three days after application (3DAA). However five days after application (5DAA) residues were similar at 7.67 mg/kg. Analysis of the retained samples on 5DAA confirmed the results from the first analysis.

In honeybee larvae samples, residues of 2,4-D were determined for all hives during bee exposure. Three days after application (3DAA) mean residues were 2.41 mg/kg, declining to 0.98 mg/kg five days after application (5DAA).

RMS comment:

The study report was of a good quality, with the methodology and results clearly presented. As required by OEPP/EPPO (2010) the objective of the study was clearly defined as measuring the residues of the test item in various honeybee matrices after application to a flowering crop. The crop used was of known high attractiveness to honeybees and was in flower at the time of application, ensuring maximal exposure to foraging bees. It is noted that both the tunnel size and colony (hive) numbers were in excess of those recommended by the referenced guideline. However, given the

residue measurements as primary endpoints in the test, this approach seems logical to ensure samples of adequate size to detect the test item residues. Likewise, pre-treatment sampling is deemed appropriate to provide background residue levels, as opposed to running a separate concurrent control group.

Application during active bee foraging was confirmed in the study, with ≥ 20 bees foraging/m² observed (target ≥ 5 /m² according to OEPP/EPPO (2010)).

Overall the study is considered to be acceptable and the results can be referenced with confidence. However it is noted that the test item utilised (2, 4-D) is not that under assessment in this EU active substance review. As such any use of this study is limited to supporting information. The applicant provided the following justification for conduction of the study:

2,4-D is a phenoxy herbicide, as is mecoprop-P. Since the two substances are closely related, the data on exposure may be used to calculate the exposure of hives to residues of mecoprop-P for determination of the rates to be used in the bee brood study.

As such it is proposed by the evaluator that the results of the referenced bee brood study (Frank, M. (2013) are relevant for any risk assessment to bees, with the above study only relevant as preliminary work for the brood study itself to justify the tested doses. Application to the flowering crop in this study at 1 x 750 g a.s./ha resulted in the following maximum residues:

- Pollen: 75.3 mg a.s./kg
- Nectar: 2.45 mg a.s./kg
- Larvae: 2.41 mg a.s./kg

Report:	CA 8.3.1.4/03, Franke, M. (2013)
Title	Effects of CMPP-P K 600 g/L OAI on the honeybee <i>Apis mellifera</i> L. in a bee brood study under field conditions Report No. 12 10 48 001 B
Guidelines:	Oomen PA, De Ruijter A and Van der Steen J OEPP/EPPO 22: 613-616 (1992)
GLP:	Yes
Deviations	None

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

1. **Test materials:** Mecoprop-P K 600
 - Description:** Brown liquid
 - Lot/Batch #:** 33-01-119
 - Purity:** 609.1 g Mecoprop-P/L
 - CAS #:** 66423-05-0
 - Density:** 1.254 g/mL
 - Stability of test compound:** Stable
2. **Vehicle:** Sucrose solution; 50% aqueous

Positive control:	Insegar 25WG – 250 g/kg fenoxycarb
3. Test animals	
Species:	Honey bee (<i>Apis mellifera carnica</i> P.)
Age:	Queen bees bred in 2011
Source:	Bee-keeper Mr Kern, Leipzig/Rehbach, Germany
Acclimatisation period:	4 days
Diet:	Free access to untreated natural nectar and pollen sources. No artificial food was offered other than the applied dose of control/test/reference item.
Housing:	Free standing hives under natural field conditions; each hive was a small colony of 11 combs; 4-8 brood combs and 6-11 combs with food
Starting populations:	Estimated via the assumption that a “Deutsch Normalmass” comb with an area of 825.1 cm ² could be covered in maximum by 900 bees per comb side. Therefore the maximum number of bees per colony consisting of one super with in total 11 combs and two bounding hive walls could be theoretically 21600 (19800 bee on combs and 1800 bees on walls). Starting colony size = 7988 - 10913

B. STUDY DESIGN AND METHODS

1. In life dates:

30 July – 29 August 2012

2. Animal assignment and treatment

Healthy small bee colonies with one body containing 11 combs, including 4-8 brood combs with all brood stages present and 6-11 combs with food were selected for the study. Colonies were assigned to one of 4 treatment groups: control, 3.75 g a.s./hive, 0.150 g a.s./hive and reference item, with 3 colonies per treatment group. Bees were exposed to the treatment via feeding directly into the hives with a single treatment of 1 litre of treated or untreated sucrose solution at each colony.

3. Dose selection

The test item low dose equivalent to 0.150 g a.s./hive was calculated to represent maximum worst case residues as measured in a surrogate phenoxy herbicide (study summarised under CA 8.3.1.4/01). In this study a maximum test item concentration in pollen and larvae was measured as ~ 75 mg a.s./kg food. As the proposed GAP for the representative use of mecoprop-P is double that applied to plants in the pre-cursor residues study (i.e. 1500 g a.s./ha versus the 750 g a.s./ha applied in Mack, P. (2012)) then the dose concentration in the treated food source should also be doubled = 150 g a.s./L sucrose solution)

Test item high dose level equivalent to 3.75 g a.s./hive represents the in-use concentration of the formulated spray material (high volume spray strength) and is proposed in line with the recommendations of the EPPO Bulletin 22 guidance by Ooman et al (1992). The proposed application rate of mecoprop-P is 1500 g a.s./ha, and a maximum application volume of 400L/ha is proposed. As such, according to the “high volume spray strength” calculation the concentration to be tested (and representing a theoretical 100% exposure to the in-use concentration) would be 3.75 g a.s./L.

4. Observations

Mortality of the bees was assessed daily from 3 days before to 27 days after application, respectively. Sub-lethal effects, such as changes in behaviour were monitored daily. Colony assessments (food stores, colony strength and general brood status) were carried out on Days -1, 4, 8, 15, 22 and 27. Detailed brood status assessments were carried out on Days -1, 4, 8, 15 and 22.

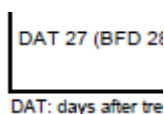
Assessments of mortality were carried out by using dead bee traps attached to the test hives. The dead bee traps were constructed in such a way, that a bee could not leave the hive carrying a dead bee or brood without dropping it into the trap. All bees leaving the hive had to exit through the dead bee trap (22 cm x 18 cm x 12 cm) covered with metal gauze lids (mesh width 1.2 cm). The dead bees dropped onto a bottom slide, which could be withdrawn in order to count and remove the bees collected in each trap. The assessments of the number of dead bees in the dead bee traps were performed daily, starting the day after placing the bee hives at the monitoring site, to provide results for a 3-day acclimatisation period.

For colony strength assessments the number of bees per control, test and reference item colony was estimated according to IMDORF et al. (1987) and IMDORF & GERIG (1999) on DAT -1, DAT 4, DAT 8, DAT 15, DAT 22 and DAT 27. The assessment based on the assumption that a “Deutsch Normalmass” comb with an area of 825.1 cm² could be covered in maximum by 900 bees per comb side. Therefore the maximum number of bees per colony consisting of one super with in total 11 combs and two bounding hive walls could be theoretically 21600 (19800 bee on combs and 1800 bees on walls).

The area of each frame side covered with the different stages of brood (including eggs, larvae and capped cells), honey, pollen and empty cells were assessed as x/8 (number of eight's) per frame side. The total brood area per colony based on a frame size of 37 cm x 22.3 cm = 825.1 cm² per comb side (total comb area per colony 18152 cm²). The originally assessed “eight's” of brood or food were transferred in cm² (taking into account that 1/8 of a comb is equivalent to 103.1 cm²). The brood assessments was conducted only if the weather situation have had no negative influence on brood development after opening the hives for assessing the brood.

During the test one full generation cycle including all brood stages was observed over one 27-day test period.

Observation Schedule:

Table B.9.3.1-10: Timeline of study phases


DAT 27 (BFD 21)

DAT: days after tre

5. Statistics

For mortality (number of dead bees/day), and brood termination rate (% terminated eggs/colony) the arithmetic mean and the standard deviation per replicate and treatment group were calculated.

Pre-treatment data were statistically evaluated using the TUKEY-test ($\alpha = 0.05$), which showed whether the treatment groups were statistically significantly different or not.

The post-treatment data were evaluated using pair-wise statistical testing methods comparing treatments separately against the control. The STUDENT-t test (for variance homogeneous data) or the WELCH-t test (for variance inhomogeneous data) were used for pair-wise comparison of treatments with the control (mortality and brood termination rate: one-sided greater).

II. RESULTS AND DISCUSSION

A. MORTALITY

Adult honeybees

The mortality of honeybees observed on the days before application was low and similar in all treatment groups, indicating comparable and well adapted colonies. After application no increase in mortality was observed for the test item low or high treatment compared to the control.

The exposure of honeybees to the reference item did not result in an increased number of dead adult bees.

Table B.9.3.1-11: Adult mean daily mortality (individuals/colony/day)

Test phase	Control	Mecoprop-P 0.150 g a.s./hive	Mecoprop-P 3.75 g a.s./hive	Ref item 0.75 g a.s./hive
Pre-exposure (d -3 to d 0)	7.6	8.2	9.9	10.6
Post-exposure (d 0 to d 27)	11.5	11.1	11.8	9.6

Larvae/pupae

No increased mortality of honeybee larvae or pupae was observed on the days before application in all treatment groups, indicating comparable, healthy and well adapted colonies. After application no increase in mortality was observed for the test item low or high treatment compared to the control.

In the reference item an increased number of dead pupae was found between Day 12 and 26.

Table B.9.3.1-12: Juvenile/Pupae mean daily mortality (individuals/colony/day)

Test phase	Control	Mecoprop-P 0.150 g a.s./hive	Mecoprop-P 3.75 g a.s./hive	Ref item 0.75 g a.s./hive
Pre-exposure (d -3 to d 0)	0.0	0.1	0.0	0.0
Post-exposure (d 0 to d 27)	0.0	0.0	0.0	8.3*

*Statistically significantly different from control group ($\alpha = 0.05$)

B. BEE BEHAVIOUR

The exposure of bees to the test item treatment did not result in behavioural abnormalities, e.g. intoxication symptoms or differences if compared to the control. Bees were calm and active.

C. COLONY STRENGTH

In all treatment groups the mean number of bees per colony was on a similar level one day before application.

In all four treatment groups the estimated number of bees per colony decreased throughout the test. At the last assessment on Day 27 the colony strength resulted to an average of 7500 bees (- 13 % versus day -1 count), 8063 bees (- 13 %), 8213 bees (- 19 %) and 4800 bees (- 51 %) in control, test item (low), test item (high) and reference, in comparison to the pre-application level. Thus, control and both test item treatments (low and high) showed a similar development, whilst the reference was significantly reduced.

D. BROOD AREA

Before treatment honeybee queens were healthy, actively laying eggs and colonies were generally producing brood in comparable amounts in all test colonies.

Overall, the total mean brood nest area (sum of comb area occupied by eggs, larvae and capped cells) in the test item treatment (low) increased during the course of the study and developed even better, when compared to the control. At the same time a decrease of the total mean brood nest area occurred in the test item treatment (high) and in the reference. The effect of the reference item on brood development showed that the test system was sensitive to detect possible effects on brood development.

Table B.9.3.1-13: Brood Development (total area as cm²)

Test phase	Control	Mecoprop-P 0.150 g a.s./hive	Mecoprop-P 3.75 g a.s./hive	Ref item 0.75 g a.s./hive
Pre-exposure (d -1)	5810	6051	7701	5621
End of post- exposure (d 27)	6369 (+9%)	7907 (+31%)	5879 (-24%)	3300 (-41%)

E. BROOD ASSESSMENT

Brood termination rate [%]

Following the assessment of single cells from the egg stage to the successfully hatched worker bee, the mean termination rate at the end of the study was 26.0, 19.0 and 24.7 % for eggs, 7.7, 4.3 and 18.7 % for young larvae and 2.7, 3.0 and 5.7 % for old larvae in the control, test item (low) and test item (high), respectively.

Overall the mean brood-termination rate in the test item treatments was not statistically significantly different to the control.

The treatment with the reference item, Insegar led to an effect on the development of the marked eggs and larvae, resulting in a mean termination rate of 64.0 % for eggs, 14.3 % for young larvae and 10.3% for old larvae.

Table B.9.3.1-14: Brood termination by life stage on D27 (%)

Brood life stage	Control	Mecoprop-P 0.150 g a.s./hive	Mecoprop-P 3.75 g a.s./hive	Ref item 0.75 g a.s./hive
Eggs	26.0	19.0	24.67	64.0
Young larvae	7.67	4.33	18.67	14.33
Old larvae	2.67	3.00	5.67	10.33
Overall	12.11	8.78	16.33	29.56

Brood-index

The progress of the brood-indices in the course of the study indicated a largely continuous brood development in the control and test item treatments. According to the termination rates the brood-indices were slightly higher in the control if compared to test item treatments. No distinct effect of the test item treatments on the brood development was identifiable following the labelling of the eggs and larval brood. The termination rate in the reference item group was also reflected by the brood-indices.

Brood compensation index

The compensation-indices showed a continuous brood development in the test item treatments, as well as in the control group. Generally the brood-compensation indices of all treatment groups were higher than the corresponding brood-indices indicating that cells with terminated brood were partially refilled with new eggs, which developed successfully.

Despite a loss of brood stages in the reference item group between Day 5 and 16 some of the emptied cells were refilled with eggs and thus the brood-compensation indices of this period were slightly higher than the corresponding brood-indices.

E. WEATHER CONDITIONS

Weather conditions were monitored from a station within 1 km of the test site. Daily temperature ranged from 0.5 – 30.0°C (mean = 19.4°C). Humidity was between 50.3-80.5% (mean = 64%). Daily precipitation was 0-15mm, with total rainfall throughout the study reported as 37mm and rainfall only on days 0, 1, 4, 22-24 and 27.

III. CONCLUSIONS

Based on the results of this study, mecoprop-P applied at a rate of 0.150 g mecoprop-P/hive does not adversely affect honey bee colonies. At a rate of 3.75 g mecoprop-P/hive there were no adverse effects on honey bee colonies, except for a slight reduction in the development of brood nest size.

RMS comments:

The study was well reported and appeared to be conducted in good adherence with the relevant test guideline; Ooman et al (1992). Control mortality did not exceed 15% for either adults or juveniles throughout the test (based on estimated colony size) and there was a clear effect of the reference item on several measured parameters. Weather conditions observed are deemed as suitable to allow for normal colony behaviour.

At the low tested dose of Mecoprop-P SL 600 g/L (0.15 g a.s./L food) there were no adverse effects on any of the measured parameters compared to the control group: Adult mortality, colony size, brood area, brood termination. This treatment group was based on the assumption of dosing matching 100% of available residues of mecoprop-P in pollen and nectar following the critical representative GAP use of 1 x 1500 g a.s./ha.

At the high tested dose of Mecoprop-P SL 600 g/L (3.75 g a.s./L food) there were no statistically significant effects on any of the measured parameters in comparison to the control group. However, it is noted that there was a definite effect on brood development area (-24% versus pre-dose area, compared to control performance of +9%), and also a noticeable increase in the % termination of young larvae (18.67% versus 7.67% in the control group). Considering the per-replicate data on these two parameters, the termination of young larvae is comparable to the control group for 2/3 replicates, while the 3rd is significantly higher (52 %). This represents the possibility that this observed effect at the concentration of 3.75 g a.s./L food was not test item related. Likewise, the total brood development area in the high dose group appears to have been adversely influenced by a single replicate (rep 1), which showed an area decrease of 62% versus the pre-exposure area. In contrast the other 2 replicates displayed a change in total brood area of – 4.8% and – 9.6%. It should be noted however that both the control and low dose group both increased their brood development area, without an outlier replicate evident.

The tested concentrations of mecoprop-P were based on either sound scientific rationale (low dose: based on maximum measured residues of a similar substance in pollen and nectar following the proposed field rate), or on the guideline recommendations (high dose: High volume spray strength calculation).

Overall it can be concluded that exposure of bee colonies to mecoprop-P at a concentration in sucrose solution of 0.15 g a.s./L does not cause any adverse effects to brood parameters after a 27-day period.

Exposure at a higher concentration of 3.75 g a.s./L sucrose solution cannot be comprehensively concluded as not causing any biologically adverse brood effects.

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

Report:	CP 10.3.2.1/01
Title:	Stevens, J (2014a) Mecoprop-P K 600 g/L – A rate-response laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphi</i> (Hymenoptera, Braconidae) Testing Laboratory: Mambo-Tox Ltd. Study Number: NUF-14-1 Date: 17 July 2014
Guideline:	Mead-Briggs et al. (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphi</i> (De Stefani-Perez) (Hymenoptera, Braconidae)
GLP:	Yes

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS**A MATERIALS****Test material**

Test item:	Mecoprop-P K 600 g/L
Description:	Brown liquid
Lot No./Batch No:	18-32-122
Active ingredient content:	587.3 g Mecoprop-P/L
Density:	1.248 g/mL
Vehicle and/or positive control	Purified water (control) Dimethoate 400 EC (positive control)

Test system

Organism (<i>Species</i>):	<i>Aphidius rhopalosiphi</i>
Age:	Adult, < 48 hr old at initiation
Source:	In house culture, established with wasps initially obtained from Katz Biotech AG, Baruth, Germany
Acclimatisation period:	None

Test environmental conditions

Temperature:	21-22°C
Humidity:	68-73%
Photoperiod:	16 hours light / 8 hours dark

Light intensity:	1114 Lux (mortality phase), 5010 (reproductive phase)
Food:	The wasps were provided with 1:3 v/v solution of honey in water on cotton wool

B STUDY DESIGN AND METHODS

In life dates: 28 May 2014 – 7 July 2014

Experimental treatment: 10 adult *Aphidius rhopalosiphi* (at least 5 females) were placed into arenas according to Mead-Briggs et al (2000) and exposed to freshly dried residues of Mecoprop-P K 600. The test units consisted of glass plates fitted to a square frame, each wall containing three holes covered with fine-gauge mesh, for ventilation. The complete units were held together with elastic bands. To prevent a build up of any pesticide vapours and to maintain environmental conditions in the arenas, air was forced through the units using a small pump linked to one of the holes in the wall.

For the mortality phase (48 hour duration) total of 7 treatment groups were administered; control (purified water), residual exposure with mecoprop-P at rates equivalent to 2000, 1000, 500, 250 and 125 mL product/ha (1200, 600, 300, 150 and 75 g a.s./ha) and dimethoate (positive control) at 0.04 g a.s./ha. There were 4 replicates per treatment (i.e. 40 wasps in total per treatment group). The treatments were administered from directly above the glass plates using a laboratory track sprayer which had been calibrated in advance to deliver 200 L/ha spray volume.

For the reproductive phase (1 day oviposition followed by 10 days mummy development) female wasps were confined individually over pots containing approximately 15 barley seedlings (*Hordeum vulgare*) upon completion of the 48 hour mortality test. The plants were untreated and had been infested with host aphids prior to the test. The wasps were confined over the pots using clear cylinders with tops being covered in nylon netting for ventilation. After 24 hours all females were removed at the vessels maintained for a further 10 days prior to fecundity assessment.

Observations: The condition of the wasps was assessed at approximately 2, 24 and 48 hours after their introduction to the glass plates. They were classed as being:

Live	alive and apparently unaffected
Affected	upright, attempting to walk but with reduced coordination or inactive
Moribund	on their back or side, twitching slightly
Dead	not moving

At 48 hours, any moribund wasps were included with the dead insects for calculations of the percentage mortality.

For the reproduction assessment the surviving females

selected at random to be used in the test were kept under the specified conditions for 24 hours, after which time they were removed. The reproductive vessels were retained for a further 10 days, after which the number of mummies that developed in each was recorded.

Statistics:

In order to determine an LR_{50} value (median lethal rate) a Probit regression analysis was performed on the 48-hour mortality data. A Chi-square goodness of fit test ($\alpha = 0.05$) was then performed on the Probit line.

In addition the mortality in each treatment at 48 hours was compared to that in the control using Fisher's Exact Test ($\alpha = 0.05$).

For the data from the reproduction assessments a square root transformation was carried out prior to further analysis. Checks were made on the normality of the datasets from each treatment (Shapiro-Wilk test, $\alpha = 0.05$) and the homogeneity of the variance (Levene's test, $\alpha = 0.05$). Since the latter was found to be statistically significant, the data from individual treatments were compared to the control by t-test for independent samples ($\alpha = 0.05$).

Deviations from study plan:

None

II. RESULTS AND DISCUSSION**Biological Results:**

Mortality results are shown in Table B.9.3.2-01 below.

Table B.9.3.2-01: Mortality of *Aphidius rhopalosiphi* after 48 hours of exposure

Treatment	Rate (mL product/ha)	% mortality ^{a)}	Corrected % mortality ^{b)}
Control	-	2.5	-
Mecoprop-P K 600 g/L	2000	100*	100
	1000	80.0*	79.5
	500	10.0	7.7
	250	0.0	0.0
	125	0.0	0.0
Toxic reference	-	100*	100

^{a)} Results for individual treatments compared to control using Fisher's Exact Test ($\alpha = 0.05$). Values marked with an asterisk (*) differed significantly.

^{b)} Calculated using Abbott's formula.

Probit analysis of the results determined the 48-hour LR_{50} for Mecoprop-P K 600 to be 762.1 mL product/ha (447.6 g a.s./ha).

The mean number of mummies produced per surviving female was 30.1 in the control treatment, compared with 33.0, 32.7, and 26.6 in the 500, 250 and 125 mL product/ha (300, 150 and 75 g a.s./ha) treatment rates of Mecoprop-P K 600 g/L, respectively. Reproductive results are shown in the

below table:

Table B.9.3.2-02: Reproduction capacity of *Aphidius rhopalosiphi* after 10 days of exposure

Treatment	Rate (mL product/ha)	Mean number mummies per female ^{a)}	Effect on reproduction % ^{b)}
Control	-	30.1	-
Mecoprop-P K 600 g/L	500	33.0	-9.5
	250	32.7	-8.5
	125	26.6	11.7

a) Individual treatments compared to control by t-test ($\alpha = 0.05$). No significant differences observed.

b) A positive value indicates a decrease in reproduction, a negative value an increase (relative to the control).

Validity: The following validity criteria were specified in the study plan:

- Control treatment mortality < 13% = 2.5%
- Toxic reference treatment mortality > 50% = 100% at the recommended rate of 0.1 mL product/ha
- Mean number of mummies in the control treatment > 5.0 per female = 30.1 mummies/female
- No more than 2 control females should produce zero mummies = 0/15 produced zero mummies.

All validity criteria were met in accordance with the referenced guideline.

III. CONCLUSIONS

The 48-hour LR₅₀ was found to be >762.1 mL product/ha (447.6 g a.s./ha). The NOER for mortality was considered to be 500 mL product/ha (293.7 g a.s./ha). The reproductive performance of surviving wasps was not significantly affected by Mecoprop-P K 600 g/L at treatment rates of up to and including 500 mL product/ha (293.7 g a.s./ha).

RMS comments:

The study was well reported and conducted in good adherence with the Mead-Briggs (2000) study guideline. All validity criteria were met and study methodology was as recommended. The study is considered valid and acceptable for use in the risk assessment.

The agreed study endpoints are a 48-hour LR₅₀ = 762.1 mL product/ha, equivalent to 447.6 g a.s./ha. < 50% effects on reproduction were seen at 500 mL product/ha, equivalent to 293.7 g a.s./ha.

Endpoints expressed in terms of active substance are derived using the measured batch content of mecoprop-p (587.3 g/L).

Report:	CP 10.3.2.1/02
Title	Fallowfield, L (2014) Mecoprop-P K 600 g/L – A rate-response laboratory bioassay of the effects of fresh residues on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) Testing Laboratory: Mambo-Tox Ltd. Study Number: NUF-14-2 Date: 26 June 2014
Guideline:	Blümel <i>et al.</i> (2000). Laboratory residual contact test with the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products.
GLP:	Yes

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A MATERIALS

Test material

Test item:	Mecoprop-P K 600 g/L
Description:	Brown liquid
Lot No./Batch No:	18-32-122
Active ingredient content:	587.3 g Mecoprop-P/L
Density:	1.248 g/mL
Vehicle and/or positive control	Purified water (control) Dimethoate 400 EC (positive control)

Test system

Organism (<i>Species</i>):	<i>Typhlodromus pyri</i>
Age:	Protonymphs < 24 hours old at initiation
Source:	In house culture, established with mites initially obtained from P.K. Nützlingszuchten, Welzheim, Germany

Test Environmental conditions

Temperature:	25 - 27°C
Humidity:	68 - 90%
Photoperiod:	16 hours light / 8 hours dark

Light intensity: 550 – 1400 Lux

Food: Pollen (apple:almond 1:1) was provided as food.

B STUDY DESIGN AND METHODS

In life dates: 07 May 2014 – 16 June 2014

Experimental treatment: 20 protonymphal *Typhlodromus pyri* mites were placed on glass plates and exposed to freshly dried residues of Mecoprop-P K 600. The test arenas consisted of glass plates formed from two microscope slide cover slips. An oblong ring of “Non-Drying Insect Glue” was drawn onto each plate to make an arena in which to confine mites. The overall arena design was in accordance with the ‘open method’ described in Blumel et al (2000).

Three treatment groups were administered; control (purified water), residual exposure with Mecoprop-P at rates equivalent to 2500, 1250, 625, 312.5 and 156.25 mL product/ha (1500, 750, 375, 187.5 and 93.75 g a.s./ha) and dimethoate (positive control) at 6 g a.s./ha. There were 3 replicates per treatment (i.e. 60 mites in total per treatment group). The treatments were administered prior to organism addition using a laboratory track sprayer which had been calibrated in advance to deliver 200 L/ha volume.

Observations: The condition of the mites was assessed with the aid of a binocular microscope at 1 and 7 days after treatment (DAT). They were recorded as being:

Alive	still moving
Dead	no sign of movement
Stuck	embedded in the sticky barrier
Drowned	dead on the water source
Missing	not visible

Any dead, drowned or stuck mites were removed at the time of each assessment.

The total egg production (numbers of eggs plus live and dead juvenile stages) was recorded for each unit over Days 7-14. Assessments of oviposition activities were carried out at 9, 11 and 14 DAT. Any eggs and nymphs present were recorded and then removed. In addition, the condition of the adult female and male mites in each arena was recorded.

Statistics: In order to determine the NOER for mortality, the percentage in each treatment was compared to the control using Fisher’s Exact Test ($\alpha = 0.05$).

To determine the NOER for reproduction, the results for eggs per female in each replicate were compared. The data were checked for normality (Shapiro-Wilk test, $\alpha = 0.05$) and for homogeneity of variance (Levene’s test, $\alpha = 0.05$). Finally Analysis of Variance and Dunnett’s t-test (2-sided, α

= 0.05) was used to compare treatments.

Deviations from study plan: None

II. RESULTS AND DISCUSSION

Biological Results: Mortality results are shown in Table B.9.3.2-03.

Table B.9.3.2-03: Mortality of *T. pyri*. after 7 days of exposure

Treatment	Application rate (mL product/ha)	Mean % mortality ^{a)} at 7 DAT	Corrected % mortality ^{b)} at 7 DAT
Control	-	3	-
Mecoprop-P K 600 g/L	2500	15	12
	1250	15	12
	625	8	5
	312.5	7	3
	156.25	18*	16
Toxic reference	-	82*	81

^{a)} Results for individual treatments compared to control using Fisher's Exact Test ($\alpha = 0.05$). Values marked with an asterisk (*) differed significantly.

^{b)} Calculated using Abbott's formula.

The mean number of eggs produced per female was 9.5 in the control treatment, compared with values of 6.8, 7.6, 9.2, 7.8 and 8.6 in the 2500, 1250, 625, 312.5 and 156.25 mL product/ha (1500, 750, 375, 187.5 and 93.75 g a.s./ha) treatment rates of Mecoprop-P K 600 g/L, respectively. Results are shown in Table B.9.3.2-04.

Table B.9.3.2-04: Reproduction capacity of *T. pyri*. after exposure to Mecoprop-P K 600 SC

Treatment	Application rate (mL product/ha)	Mean number of eggs per female ^{a)}	% change in reproduction relative to control ^{b)}
Control	-	9.5	-
Mecoprop-P K 600 g/L	2500	6.8 *	28.1
	1250	7.6	20.0
	625	9.2	2.5
	312.5	7.8	18.0
	156.25	8.6	8.9

^{a)} Results for reproduction were compared by one-way ANOVA and Dunnett's t-test ($\alpha = 0.05$). Treatment means that differed significantly from the control are indicated with an asterisk (*).

^{b)} Percentage change in egg production, relative to the control. A positive value indicates a decrease.

Validity: The following validity criteria were specified in the study plan:

- Control treatment mortality < 20% = 3%
- Toxic reference treatment mortality > 50% at 8-15 mL reference product/ha = 82% at 15 mL/ha
- Mean number of eggs in the control treatment ≥ 4.0 per female = 9.5 eggs/female.

All validity criteria were met.

III. CONCLUSIONS

The 7-day LR_{50} was found to be >2500 mL product/ha (>1468 g a.s./ha), the maximum tested. The NOER for mortality was considered to be 2500 mL product/ha (1468 g a.s./ha). The reproductive performance of surviving mites was not significantly affected by Mecoprop-P K 600 g/L at treatment rates of up to and including 1250 mL product/ha (734 g a.s./ha). Less than 50% effects on reproduction were seen up to and including 2500 mL/product/ha (1468 g a.s./ha)

RMS Comments:

The study was well reported and conducted in good adherence with the Blumel et al (2000) study guideline. All validity criteria were met and study methodology was as recommended. The study is considered valid and acceptable for use in the risk assessment.

The agreed study endpoints are a 7-day LR_{50} = >2500 mL product/ha, equivalent to 1468 g a.s./ha. < 50% effects on reproduction were seen at 2500 mL product/ha, equivalent to 1468 g a.s./ha.

Endpoints expressed in terms of active substance are derived using the measured batch content of mecoprop-p (587.3 g/L).

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA**B.9.4.1. Earthworm – sub-lethal effects**

Report:	CA 8.4.1/01, Stojanowitsch, M. (2014)
Title	Mecoprop-P TGAI: sublethal toxicity to the earthworm <i>Eisenia fetida</i> Michaelson (Haplotaxida, Lumbricidae) in artificial soil Report No. S13-00246
Guidelines:	OECD 222 (2004) ISO Guideline 11268-2 (1998)
GLP:	Yes
Deviations	No major deviations

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS**A. MATERIALS**

1. **Test materials:** Mecoprop-P TGAI
Description: Light yellow solid
Lot/Batch #: 3860
Purity: 92.63 % w/w
CAS #: 16484-77-8
Stability of test compound: Stable
2. **Vehicle and/or positive control** Solvent: acetone
Positive control: Carbendazim (separate study)
3. **Feed:** Dried cow manure
4. **Test animals**
Species: *Eisenia fetida* ssp. *andrei*
Age: >2 months, <1 year
The age of the worms used for the test did not differ by more than 4 weeks.
Weight: 316 – 600 mg at initiation
Source: In-house
Acclimatisation period: 2 days
5. **Environmental conditions**
Temperature: 19.2 – 20.3 °C
pH: Day 0: 5.5
Day 56: 6.1-6.3

Substrate:	Artificial according to OECD 207 (10 % peat content), maintained at 25.0 – 27.8 % water content.
Photoperiod:	16 hours light/8 hours dark, 400-800 lux

B. STUDY DESIGN AND METHODS

1. In life dates:

17 April – 15 November 2013

2. Experimental treatments

The objective of the study was the assessment of the effects of mecoprop-P TGAI on the mortality, body weight change and reproductive output of the earthworm *Eisenia fetida*. The test organisms were kept in samples of a defined artificial soil with 10 % peat content to which 5 different concentrations of mecoprop-P (3.3, 6.0, 10.8, 19.4 and 35.0 mg a.s./kg soil dry weight) had been applied by mixing.

The test item was dissolved in acetone to prepare stock solutions which were then applied to quartz sand. After evaporation of the solvent, the sand was mixed with the pre-moistened artificial soil. Final moistening of the soil to 55 % of the WHCmax was conducted during the application. After the application, the test substrate was filled into the replicate vessels. Ten adult worms from a synchronised culture were exposed per replicate vessel (500 g dry mass of artificial soil). A solvent control (acetone) was included in the test. Four replicates were used for each test item group and 8 replicates for the acetone control group.

Four weeks after the application, the adult worms were removed from the test units and mortality as well as biomass development was assessed. The effects on reproduction were assessed after further 4 weeks by counting the juveniles extracted from the test soil using a heated water bath. The effects of the test item on the reproduction rate were determined by comparison with the control group. A toxic reference item was tested regularly as a separate study to confirm the sensitivity of the test organisms. Vessels were fed on days 7, 14, 21 and 28 of the test with 4 g dried cow manure.

3. Observations

Body weight was recorded on Day 0 and Day 28. Mortality, abnormal behaviour and pathological symptoms of the introduced adult worms were recorded after 28 days. The number of juveniles per replicate was recorded on Day 56. Soil pH and water content was measured on days 0 and 56.

II. RESULTS AND DISCUSSION

A. FINDINGS

During exposure of the test organisms to mecoprop-P, no unusual behavioural effects were observed. After 28 days of exposure, one earthworm was found dead at 6.0 mg a.s./kg soil dry weight.

A summary of the effects of mecoprop-P on mortality, bodyweight change and juvenile product is shown in Table B.9.4.1-01 below.

Table B.9.4.1-01: Effects of mecoprop-P TGAI on *Eisenia fetida* ssp. *andrei* mortality, body weight change and reproduction

	Concentration of mecoprop-P TGAI [mg/kg soil dry weight]					
	Solvent control	3.3	6.0	10.8	19.4	35.0
Mean mortality [%]	0.0	0.0	2.5	0.0	0.0	0.0
Mean weight change [%]	+27.3	+23.2	+27.5	+19.9	+17.8	+24.7
Juveniles/replicate (mean)	306.8	282.8	298.3	283.0	233.3*	242.5*
Reproduction [%] (deviation from control)	--	-7.8	-2.8	-7.8	-24.0	-21.0
Endpoints [mg/kg soil dry weight]						
NOEC _{reproduction}	10.8					
LOEC _{reproduction}	19.4					
EC ₁₀ _{reproduction}	9.0 (lower confidence limit: 5.1, upper confidence limit 12.8)					
EC ₂₀ _{reproduction}	26.4 (lower confidence limit: 18.2, upper confidence limit 52.4)					
EC ₅₀ _{reproduction}	n.d.					

* Statistically significantly different from the control (Williams test, descending order, $p \leq 0.05$)

n.d. could not be determined

Validity criteria were met in accordance with OECD 222 as follows:

- Control adult mortality must not exceed 10% = 0%
- Mean control replicate juvenile production must be at least 30 = 306.8 (range = 243 – 356)
- Coefficient of variation for control group juvenile production must not exceed 30% = 12.8%

All validity criteria were achieved.

In the most recent toxic reference study with carbendazim, there was a statistically significant effect on reproduction at a concentration of 5.0 mg a.s./kg soil dry weight.

III. CONCLUSIONS

No effects on mortality or weight change were detected.

The NOEC for reproduction of *Eisenia fetida* ssp. *andrei* was 10.8 mg/kg soil dry weight and the LOEC for reproduction was 19.4 mg/kg soil dry weight.

The EC₁₀ and EC₂₀ for reproduction were calculated as 9.0 and 26.4 mg a.s./kg soil dry weight, respectively. The EC₅₀ for reproduction was >35.0 mg a.s./kg soil dry weight.

RMS comments:

The study was well reported and conducted in good adherence with the referenced guidelines. It is noted that no parallel untreated control group was tested, which is usually done to ensure no adverse effects caused by the solvent vehicle alone. However, as the solvent control group comfortably met all validity criteria, and no behavioural or clinical effects were seen, the solvent can be assumed to have been adequately evaporated so not as to cause toxic symptoms in the organism. The results of a separate toxic reference study using worms from the same culture stock confirm adequate sensitivity of the test system: Significant reproductive effects were seen at 5 mg a.s./kg, which is within the 1-5 mg a.s./kg range advised in OECD guideline 222. Overall the study is considered valid and acceptable for risk assessment use.

The agreed endpoints are:

56-day NOEC = 10.8 mg a.s./kg soil dry weight

56-day EC₁₀ = 9.0 mg a.s./kg soil dry weight

56-day EC₂₀ = 26.4 mg a.s./kg soil dry weight

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

Data with the representative formulation have been submitted in order to address this data requirement. Please refer to study summaries included in Volume 3 (CP) point B.9.7.2.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

Ref.: IIA. 8.5; IIIA. 10.7.1. Todt, 1989 b: Effects of the plant protective agent Duplosan KV on the activity of the microflora of soil. 2. Nitrification.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The effects of Duplosan KV on soil micro-organisms which convert NH₄ into NO₃ (and NO₂) was studied by comparing the rate of nitrification of added (NH₄)₂SO₄ with and without Duplosan KV according to the BBA guideline VI 1-1.

Duplosan KV was applied to two soils a sand and a loam in triplicates of 100 g soil adjusted to 50% of maximum water capacity at 2.7 µl Duplosan KV/kg soil (corresponding to practical amount of application) and 13.7 µl Duplosan KV/kg soil, corresponding to 5 times to practical amount of application. To all soil samples, 43 mg ammonium sulphate (NH₄)₂SO₄ /100 g soil corresponding to ~9 mg NH₃-nitrogen/100 g, was added. Soil samples were taken after 7, 14, 21 and 28 days. The nitrification was determined by measuring the formation of nitrate (and nitrite). Each sample was assessed in triplicate.

Table B.9.5-01: Soil characteristics

Soil	pH	Sand %	Silt %	Clay %	Org.C %	N(min) mg/100 g soil	Microbial biomass mg C/100 g soil
1: Sand	6.7	60	32	8	0.8	0.6	19
2: Loam	6.0	68.4	29.0	2.6	1.01	4.0	41

After the addition of ammonium sulfate, the sand contained 10.0 mg $\text{NH}_4^+\text{-N}$ /100 g soil and the total of 10.6 mg N/100 g soil. The loam contained 9.2 mg $\text{NH}_4^+\text{-N}$ /100 g soil and the total of 13.2 mg N/100 g soil.

Results

In soil 1, no influence of Duplosan KV on nitrification could be detected. In soil 2, the 5-fold concentration caused a slight retardation of nitrification in the first two weeks but the nitrification was comparable to control after 3 weeks.

Table B.9.5-02: Nitrification, mean and standard deviation in mg N/100 g soil.

Soil	Treatment	Measured N	mg N/100 g soil after day			
			7	14	21	28
Sand	Control	$\text{NH}_4^+\text{-N}$	7.4	1.3	<0.1	0.1
		$\text{NO}_2^- + \text{NO}_3^-\text{N}$	4.0	10.3	12.2	12.3
		N_{total}	11.4	11.6	12.2	12.3
	Duplosan KV, 1X	$\text{NH}_4^+\text{-N}$	7.4	2.8	<0.1	<0.1
		$\text{NO}_2^- + \text{NO}_3^-\text{N}$	3.1	8.5	11.7	12.1
		N_{total}	10.5	11.3	11.7	12.1
	Duplosan KV, 5X	$\text{NH}_4^+\text{-N}$	8.5	5.0	0.6	<0.1
		$\text{NO}_2^- + \text{NO}_3^-\text{N}$	2.7	6.2	11.6	12.4
		N_{total}	11.2	11.2	12.2	12.4

Table B.9.5-03: Nitrification, mean and standard deviation in mg N/100 g soil.

Soil	Treatment	Measured N	mg N/100 g soil after day			
			7	14	21	28
Loam	Control	$\text{NH}_4^+\text{-N}$	3.8	0.1	<0.1	0.1
		$\text{NO}_2^- + \text{NO}_3^-\text{N}$	8.7	12.4	12.4	13.4
		N_{total}	12.5	12.5	12.4	13.4
	Duplosan KV, 1X	$\text{NH}_4^+\text{-N}$	4.2	0.7	<0.1	<0.1
		$\text{NO}_2^- + \text{NO}_3^-\text{N}$	8.7	11.8	13.5	13.5
		N_{total}	12.9	12.5	13.5	13.5
	Duplosan KV, 5X	$\text{NH}_4^+\text{-N}$	5.2	2.4	<0.1	<0.1
		$\text{NO}_2^- + \text{NO}_3^-\text{N}$	8.3	11.1	13.8	13.8
		N_{total}	13.5	13.5	13.8	13.8

Comments

The study is acceptable. The nitrification was inhibited for the first two weeks in the sand with the lowest microbial biomass. The nitrification was comparable to control after 28 days indicating that Duplosan KV had no long term effects on the nitrification at the two tested concentrations. The tested concentrations corresponded to 2.7 µl/kg (~2 l/ha, 5 cm soil, density 1.5 => 2000/750 = 2.7 µl/kg).

RMS comments (renewal):

The RMS has revisited the study report for the purposes of active substance renewal. The report detail is lacking in key areas such as environmental conditions during the study, the test item properties including batch, active substance content and expiry date. There was also no toxic reference group tested in tandem to demonstrate adequate test system response. No calculation of control replicate variation or nitrogen transformation rate per sampling period is made in the study report. As such the study is not considered as valid for use in regulatory risk assessment.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS**B.9.6.1. Summary of screening data**

No Screening data provided. As a herbicide, mecoprop-P was tested in a range of tier 2 terrestrial non-target plant studies. This is in line with active substance data requirements 283/2013 which states: *“For assessment of active substances with herbicidal or plant growth regulatory activity screening data shall not be used.”*

B.9.6.2. Testing on non-target plants

Report:	CA 8.6.2/01, Eley, R. (2009a)
Title	Nufarm R(+)MCCP herbicide: evaluation of the phytotoxicity and effect on seedling emergence and growth of terrestrial non target plants Report No. ACE-09-031
Guidelines:	OECD 208 OPPTS 850.4225
GLP:	Yes (except soil analysis)
Deviations	None

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS**A. MATERIALS**

1. **Test materials:** Nufarm R(+)MCCP Herbicide
Description: Colourless aqueous solution
Lot/Batch #: NB 36934
Purity: 3.815 g/L
CAS #: 16484-77-8
Stability of test compound: Stable
2. **Vehicle and/or positive control:** Tap water
3. **Environmental conditions**
Temperature: 15.4 – 23.9°C
Humidity: 38.2 – 71.4 %
Photoperiod: 16 hours light/8 hours dark at > 3000 lux
Test soil: 10:10:4 mix of sterile loam:sand:course grit
Soil parameters: pH = 7.2; classification = sandy loam; organic C = 1.5%

B. STUDY DESIGN AND METHODS

1. Dates of work:

11 March – 18 May 2009

2. Experimental conditions

In a terrestrial plant study, seedling emergence and growth were assessed following pre-emergence application at 0, 0.366, 1.10, 3.29, 9.88, 29.6, 88.9, 267 and 800 g a.s./ha in 200 L water/ha to ryegrass, wheat, maize, onion, cucumber, radish, lettuce, tomato and oilseed rape. 4 seeds per pot were sown directly into soil in non-porous plastic pots. Each pot contained 4 seeds and there were 5 replicates per treatment. Details of plant species, number of seeds per pot and pot size are shown in Table B.9.6.2-01 below. All seeds were from certified sources and were untreated.

Table B.9.6.2-01: Details of Plant Species

Family	Species	Common name	Variety (source)	Seeds/pot	Pot size
Gramineae	<i>Lolium perenne</i>	Ryegrass	Herbiseed (Herbiseed ¹)	4	7x7x8 cm
Gramineae	<i>Triticum aestivum</i>	Wheat	XI 19 (Walnes ²)	4	7x7x8 cm
Gramineae	<i>Zea mays</i>	Maize/corn	Minipop (EW King ³)	4	7x7x8 cm
Liliaceae	<i>Allium cepa</i>	Onion	White Lisbon (EW King ³)	4	7x7x8 cm
Cucurbitaceae	<i>Cucumis sativa</i>	Cucumber	Gherkin National (EW King ³)	4	7x7x8 cm
Brassicaceae	<i>Raphanus sativus</i>	Radish	French Breakfast (EW King ³)	4	7x7x8 cm
Compositae	<i>Lactuca sativa</i>	Lettuce	Tom Thumb (EW King ³)	4	7x7x8 cm
Solanaceae	<i>Lycopersicon esculentum</i>	Tomato	Moneymaker (EW King ³)	4	7x7x8 cm
Brassicaceae	<i>Brassica napus</i>	Oilseed rape	Elle (Herbiseed ¹)	4	7x7x8 cm

1 Herbiseed Ltd. Twyford, RG10 0NJ, UK

2 Walnes Seeds Ltd. Framlingham, IP13 9EE, UK

3 EW King & Co. Ltd. Kelvedon, CO5 9PG, UK.

Mecoprop-P was applied to the seeds, pre-emergence in the pots by means of a calibrated track sprayer and the seeds were incubated in a glasshouse.

Assessments for emergence, plant mortality and visual injury were made 14 and 21 days after 50% emergence in the untreated controls. Plants were harvested 21 days after 50% control emergence and assessments were made for phytotoxicity, measurement of shoot length and dry weight of total live biomass per pot above the soil surface.

Further attempts were made for species which failed to achieve 70% germination at the first attempt, until 70% germination was achieved.

3. Statistics

The ER₂₅ and ER₅₀ values were calculated from final dry weight data and final emergence data using ARM version 7 software. ARM 7.0 uses simple probit – maximum likelihood estimation method with

95% confidence level. (The estimation algorithms are provided courtesy of J.J. Hubert, University of Guelph.)

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.6.2-02: Effects on emergence, mortality, visual injury, dry weight and shoot height of monocot species tested

Tested rate (g a.s./ha)	21-day emergence (%)	21-day mortality (%)	Visual injury score (%)	foliar dry weight (g)	Shoot height (cm)
<i>L.perenne</i>					
0 (control)	90	0	0	0.312	28.466
0.366	100	0	0	0.358	27.650
1.1	100	0	0	0.386	28.750
3.29	95	0	0	0.338	28.166
9.88	95	5	5	0.378	28.534
29.6	85	0	0	0.332	28.250
88.9	100	0	4	0.316	26.800
267	90	0	34	0.184*	24.632*
800	85	5	48	0.102*	20.250*
<i>T.aestivum</i>					
0 (control)	100	0	0	0.412	20.850
0.366	100	0	0	0.425	21.300
1.1	100	0	0	0.372	20.650
3.29	95	5	5	0.376	20.300
9.88	100	0	0	0.406	20.650
29.6	100	0	0	0.388	21.000
88.9	100	0	0	0.308*	21.000
267	95	0	2	0.264*	20.800
800	90	0	31	0.136*	17.650*
<i>Z.mays</i>					
0 (control)	95	0	0	0.498	29.366
0.366	95	0	0	0.500	28.684
1.1	95	0	4	0.390	26.984
3.29	90	0	2	0.406	27.366
9.88	85	0	0	0.436	29.650
29.6	90	0	4	0.424	28.500
88.9	95	0	20	0.408	28.000
267	100	0	37	0.266*	22.000*
800	95	0	45	0.218*	21.250*
<i>A.cepa</i>					
0 (control)	95	5	0	0.054	16.200
0.366	90	0	0	0.060	16.500
1.1	100	5	5	0.052	15.900
3.29	95	5	5	0.052	16.430
9.88	100	0	16	0.046	15.900
29.6	95	20	41	0.028*	10.030*
88.9	85	20	52	0.016*	8.550*
267	95	50	76	0.001*	3.866*
800	35*	20	85	0.004*	3.667*

*statistically significant difference to control group (P < 0.05)

Table B.9.6.2-03: Effects on emergence, mortality, visual injury, dry weight and shoot height of Dicot species tested

Tested rate (g a.s./ha)	21-day emergence (%)	21-day mortality (%)	Visual injury score (%)	foliar dry weight (g)	Shoot height (cm)
<i>C.sativa</i>					
0 (control)	75	0	0	1.378	13.45
0.366	80	5	10	1.390	11.68
1.1	80	0	0	1.546	13.68
3.29	65	5	28	1.048*	10.52
9.88	90	5	5	1.572	12.82
29.6	90	10	17	1.400	11.22
88.9	80	15	20	1.158	10.88
267	70	25	62	0.440*	5.916
800	55*	55	100	0.000*	0.000
<i>R.sativus</i>					
0 (control)	90	0	0	0.450	9.200
0.366	80	0	0	0.374	8.950
1.1	75	5	8	0.456	9.782
3.29	75	0	0	0.364	10.00
9.88	90	5	9	0.400	8.800
29.6	75	0	0	0.358	8.966
88.9	55*	5	17	0.270*	9.900
267	40*	20	69	0.058*	3.793*
800	45*	30	95	0.024*	0.550*
<i>L.sativa</i>					
0 (control)	70	0	0	0.162	5.802
0.366	70	0	0	0.190	5.250
1.1	70	0	14	0.134	4.684
3.29	65	0	0	0.176	5.700
9.88	75	0	21	0.130	3.934*
29.6	75	0	12	0.144	5.032
88.9	60	0	19	0.108*	4.716
267	30*	5	82	0.010*	2.300*
800	40*	0	75	0.012*	2.887*
<i>L.esculentum</i>					
0 (control)	90	0	0	0.716	12.45
0.366	80	0	2	0.714	12.07
1.1	85	5	9	0.748	11.55
3.29	75	0	0	0.714	13.43
9.88	60*	5	7	0.564	11.77
29.6	65*	0	0	0.672	12.50
88.9	65*	0	0	0.624	12.80
267	65*	5	26	0.424*	10.43
800	45*	10	51	0.242*	8.300*
<i>B.napus</i>					
0 (control)	100	5	0	0.552	7.950
0.366	85	0	2	0.500	7.416
1.1	80*	5	7	0.364*	6.532
3.29	95	0	18	0.356*	6.484
9.88	85	5	11	0.552	8.100
29.6	100	5	42	0.326*	6.000*
88.9	75*	0	46	0.198*	5.816*
267	90	5	68	0.072*	2.650*
800	90	35	92	0.018*	0.750*

*statistically significant difference to control group (P < 0.05)

Validity criteria were met in accordance with OECD guideline 208 as follows:

- Control seedling emergence was at least 70% = 70-100%
- Control plant post-emergence survival was at least 90% = 95-100%
- Control plants did not exhibit visual phytotoxic symptoms = All species scored '0' for visual injury.
- Per species, environmental conditions, growing media and test vessels were the same = Confirmed from report data.

III. CONCLUSIONS

Allium cepa (onion) was the most sensitive monocotyledon based on both final foliar dry weight and emergence. *Brassica napus* (oilseed rape) was the most sensitive dicotyledon species based on final foliar dry weight and *Brassica napus* (oilseed rape) the most sensitive dicotyledon species (lowest ER₅₀) based on foliar dry weight. NOER and ER₅₀ values per species are summarised below.

Table B.9.6.2-04: NOEC and EC₅₀ values based for dry weights and emergence

Family	Species	Dry weight		Emergence	
		NOER (g a.s./ha)	ER ₅₀ (g a.s./ha)	NOER (g a.s./ha)	ER ₅₀ (g a.s./ha)
Gramineae	<i>Lolium perenne</i>	88.9	678	800	>800
Gramineae	<i>Triticum aestivum</i>	29.6	421	800	>800
Gramineae	<i>Zea mays</i>	29.6	754	800	>800
Liliaceae	<i>Allium cepa</i>	3.29	40.9	267	267-800*
Cucurbitaceae	<i>Cucumis sativa</i>	29.6	159	267	>800
Brassicaceae	<i>Raphanus sativus</i>	29.6	82.7	29.6	410
Compositae	<i>Lactuca sativa</i>	9.88	68	88.9	498
Solanaceae	<i>Lycopersicon esculentum</i>	29.6	431	3.29	800
Brassicaceae	<i>Brassica napus</i>	0.366	19.2	800	>800

* No significant reduction at 0.267 kg a.s./ha and 65% reduction at 0.8 kg a.s./ha.

RMS comments:

The study was well reported and conducted in good adherence with the referenced guidelines. All associated validity criteria were met. Although the soil analysis was not performed to GLP, the properties reported were in line with the recommendations of OECD 208, and control performance confirmed that soil of adequate properties was used. It is noted that ER₅₀ and NOER endpoints were not calculated for all measured parameters, including mortality, visual injury and shoot height. However, considering the reported results presented above, it would appear that foliar dry weight was the most sensitive parameter for the majority of tested species, including the most sensitive; *B.napus*. This is based on consideration of the tested rates encompassing 50% inhibition versus the control group. Overall the study is considered valid and acceptable.

The agreed critical study endpoints are as follows:

Lowest ER₅₀ = 19.2 g a.s./ha (as mecoprop-P), based on foliar dry weight reduction in the most sensitive species; *Brassica napus* (oilseed rape).

Report:	CA 8.6.2/02, Eley, R. (2009b)
Title	Nufarm R(+)MCP herbicide: evaluation of the phytotoxicity and effect on vegetative vigour of terrestrial non target plants Report No. ACE-09-032
Guidelines:	OECD 227 OPPTS 850.4250
GLP:	Yes (except soil analysis)
Deviations	None

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

1. **Test materials:** Nufarm R(+)MCP Herbicide
 - Description:** Colourless aqueous solution
 - Lot/Batch #:** NB 36934
 - Purity:** 3.815 g/L
 - CAS #:** 16484-77-8
 - Stability of test compound:** Stable
2. **Vehicle and/or positive control:** Tap water
3. **Environmental conditions**
 - Temperature:** 15.1 – 23.0°C
 - Humidity:** 40.9 – 65.6 %
 - Photoperiod:** 16 hours light/8 hours dark at >3000 lux
 - Test soil:** 10:10:4 mix of sterile loam:sand:course grit with 100g slow release fertiliser added.
 - Soil parameters:** pH = 7.2; classification = sandy loam; organic C = 1.5%

B. STUDY DESIGN AND METHODS

1. Dates of work:

10 March – 06 May 2009

2. Experimental conditions

In a terrestrial plant study, vegetative vigour was assessed following application at 0.122, 0.366, 1.10, 3.29, 9.88, 29.6, 88.9, 267 and 800 g a.s./ha in 200 L water/ha to ryegrass, wheat, maize, onion, cucumber, radish, lettuce, tomato and oilseed rape. Seeds were germinated in compost and then transplanted into soil in non-porous plastic pots. Each pot contained 4 plants and there were 5

replicates per treatment. Details of plant species, number of seeds per pot and pot size are shown in the below table. All seeds were from certified sources and were untreated.

Table B.9.6.2-05: Details of Plant Species

Family	Species	Common name	Variety (source)	Plants/pot	Pot size
Gramineae	<i>Lolium perenne</i>	Ryegrass	Herbiseed (Herbiseed ¹)	4	7x7x8 cm
Gramineae	<i>Triticum aestivum</i>	Wheat	XI 19 (Walnes ²)	4	7x7x8 cm
Gramineae	<i>Zea mays</i>	Maize/corn	Minipop (EW King ³)	4	7x7x8 cm
Liliaceae	<i>Allium cepa</i>	Onion	White Lisbon (EW King ³)	4	7x7x8 cm
Cucurbitaceae	<i>Cucumis sativa</i>	Cucumber	Gherkin National (EW King ³)	4	7x7x8 cm
Brassicaceae	<i>Raphanus sativus</i>	Radish	French Breakfast (EW King ³)	4	7x7x8 cm
Compositae	<i>Lactuca sativa</i>	Lettuce	Tom Thumb (EW King ³)	4	7x7x8 cm
Solanaceae	<i>Lycopersicon esculentum</i>	Tomato	Moneymaker (EW King ³)	4	7x7x8 cm
Brassicaceae	<i>Brassica napus</i>	Oilseed rape	Elle (Herbiseed ¹)	4	7x7x8 cm

¹ Herbiseed Ltd. Twyford, RG10 0NJ, UK

² Walnes Seeds Ltd. Framlingham, IP13 9EE, UK

³ EW King & Co. Ltd. Kelvedon, CO5 9PG, UK.

Mecoprop-P was applied to the plants in the pots by means of a calibrated track sprayer and the pots were maintained in a glasshouse. All plants were at growth stages BBCH 12-14 at the time of application.

Assessments for plant mortality and visual injury were made 14 and 21 days after application. Plants were harvested 21 days after application and assessments were made for phytotoxicity, measurement of shoot length and try weight of total live biomass per pot above the soil surface. Watering was performed as required.

3. Statistics

The ER₂₅ and ER₅₀ values were calculated from final dry weight data and final emergence data using ARM version 7 software. ARM 7.0 uses simple probit – maximum likelihood estimation method with 95% confidence level. (The estimation algorithms are provided courtesy of J.J. Hubert, University of Guelph.)

II. RESULTS AND DISCUSSION

A. FINDINGS

Table B.9.6.2-06: Effects on mortality, visual injury, dry weight and shoot height of monocot species tested

Tested rate (g a.s./ha)	21-day mortality (%)	Visual injury score (%)	foliar dry weight (g)	Shoot height (cm)
<i>L.perenne</i>				
0 (control)	0	0	0.780	32.35
0.122	0	0	0.698	35.45
0.366	0	0	0.772	35.35
1.1	0	0	0.756	35.90
3.29	0	0	0.792	35.00
9.88	0	0	0.766	33.50
29.6	0	2	0.706	35.20
88.9	0	4	0.666	32.90
267	0	4	0.528*	30.60
800	0	11	0.546*	32.05
<i>T.aestivum</i>				
0 (control)	0	0	2.046	37.45
0.122	0	0	1.966	37.85
0.366	0	0	2.176	38.7
1.1	0	0	2.140	38.4
3.29	0	0	2.148	38.55
9.88	0	0	2.122	38.9
29.6	0	0	2.046	38.00
88.9	0	3	2.096	38.30
267	0	0	2.136	36.75
800	0	6	1.752	35.85
<i>Z.mays</i>				
0 (control)	0	0	2.608	59.55
0.122	0	0	2.350	58.75
0.366	0	0	2.290	57.65
1.1	0	4	1.680*	51.80*
3.29	0	2	2.430	58.60
9.88	0	4	1.724*	51.90*
29.6	0	20	1.432*	47.05*
88.9	0	38	1.468*	43.85*
267	0	50	1.078*	37.50*
800	0	58	0.892*	36.40*
<i>A.cepa</i>				
0 (control)	0	0	0.324	21.90
0.122	0	0	0.328	22.35
0.366	0	2	0.396	23.25
1.1	0	0	0.374	23.25
3.29	0	0	0.446	25.30
9.88	0	0	0.352	22.35
29.6	0	2	0.320	22.75
88.9	0	14	0.286	22.30
267	0	38	0.218	22.35

Tested rate (g a.s./ha)	21-day mortality (%)	Visual injury score (%)	foliar dry weight (g)	Shoot height (cm)
800	0	61	0.126*	19.25*

*statistically significant difference to control group (P < 0.05)

Table B.9.6.2-07: Effects on mortality, visual injury, dry weight and shoot height of Dicot species tested

Tested rate (g a.s./ha)	21-day mortality (%)	Visual injury score (%)	foliar dry weight (g)	Shoot height (cm)
<i>C.sativa</i>				
0 (control)	0	0	3.718	22.75
0.122	0	0	3.148*	21.65
0.366	0	4	3.402	22.05
1.1	0	18	2.836*	19.25
3.29	0	31	2.912*	19.90
9.88	0	44	2.902*	20.90
29.6	0	67	1.782*	15.00*
88.9	60	94	0.556*	5.000*
267	100	100	0.000*	0.000*
800	100	100	0.000*	0.000*
<i>R.sativus</i>				
0 (control)	0	0	1.486	15.40
0.122	0	0	1.282	14.00
0.366	0	0	1.408	15.85
1.1	0	2	1.346	16.20
3.29	0	20	1.418	14.90
9.88	5	34	1.186	15.50
29.6	15	75	0.730*	10.85*
88.9	35	87	0.612*	8.100*
267	50	93	0.480*	4.950*
800	85	97	0.558*	1.150*
<i>L.sativa</i>				
0 (control)	0	0	1.704	7.300
0.122	0	0	1.704	8.000
0.366	0	11	1.344	7.350
1.1	0	6	1.580	7.050
3.29	0	8	1.570	7.300
9.88	0	6	1.726	7.400
29.6	0	14	1.106*	7.250
88.9	0	20	1.378	7.750
267	0	34	1.048*	7.650
800	30	80	0.582*	4.150*
<i>L.esculentum</i>				
0 (control)	0	0	3.240	23.10
0.122	0	0	2.576	21.10
0.366	0	12	2.042*	20.35
1.1	0	12	2.498	21.30
3.29	0	2	3.228	25.55
9.88	0	8	2.534	23.10
29.6	0	28	2.108*	21.15
88.9	0	48	1.354*	18.30*
267	0	66	1.016*	14.85*
800	30	91	0.740*	8.650*

Tested rate (g a.s./ha)	21-day mortality (%)	Visual injury score (%)	foliar dry weight (g)	Shoot height (cm)
<i>B.napus</i>				
0 (control)	0	0	3.274	16.95
0.122	0	0	3.180	16.65
0.366	0	0	2.848	17.50
1.1	0	2	2.348*	15.70
3.29	0	2	2.622*	16.20
9.88	0	8	2.180*	15.85
29.6	0	42	2.120*	16.35
88.9	0	60	1.386*	13.70*
267	0	69	1.240*	10.45*
800	20	84	1.372*	6.750*

*statistically significant difference to control group (P < 0.05)

Validity criteria were met in accordance with OECD guideline 227 as follows:

- Control seedling emergence was at least 70% = Not reported, considered none essential criteria as plants are pre-grown prior to use in test.
- Control plant survival was at least 90% = 100% for all species
- Control plants did not exhibit visual phytotoxic symptoms = 0% score for all species
- Per species, environmental conditions, growing media and test vessels were the same = Confirmed from report data.

III. CONCLUSIONS

Zea mays (maize) was the most sensitive monocotyledon and *Cucumis sativa* (cucumber) the most sensitive dicotyledon species based on final foliar dry weight. NOER and ER₅₀ values for all species are summarised below.

Table B.9.6.2-08: NOER and ER₅₀ values based on final dry weights

Family	Species	NOER (g a.s./ha)	ER ₅₀ (g a.s./ha)
Gramineae	<i>Lolium perenne</i>	88.9	>800
Gramineae	<i>Triticum aestivum</i>	800	>800
Gramineae	<i>Zea mays</i>	0.366	126
Liliaceae	<i>Allium cepa</i>	267	881
Cucurbitaceae	<i>Cucumis sativa</i>	<0.122	19.9
Brassicaceae	<i>Raphanus sativus</i>	9.88	89.2
Compositae	<i>Lactuca sativa</i>	9.88	459
Solanaceae	<i>Lycopersicon esculentum</i>	0.122	89.7
Brassicaceae	<i>Brassica napus</i>	0.366	129.2

RMS comments:

The study was well reported and conducted in good adherence with the referenced guidelines. All associated validity criteria were met, with the exception of data on the emergence of the seed batches

used. However, as plants are pre-grown prior to use, and all other control performance criteria were met, this is not considered to be detrimental to the validity of the study. Although the soil analysis was not performed to GLP, the properties reported were in line with the recommendations of OECD 227, and control performance confirmed that soil of adequate properties was used. It is noted that ER₅₀ and NOER endpoints were not calculated for all measured parameters, including mortality, visual injury and shoot height. However, considering the reported results presented above, it would appear that foliar dry weight was the most sensitive parameter for the majority of tested species, including the most sensitive; *C.sativa*. This is based on consideration of the tested rates encompassing 50% inhibition versus the control group. Overall the study is considered valid and acceptable.

The agreed critical study endpoints are as follows:

Lowest ER₅₀ = 19.9 g a.s./ha (as mecoprop-P), based on foliar dry weight reduction in the most sensitive species; *Cucumis sativa* (cucumber).

Reference

Frank P (2001a): BAS 037 32 H. A toxicity test to determine the effects of the test item on seedling emergence of terrestrial plants. Staatliche Lehr- und Forschungsanstalt für Landwirtschaft (SLFA), Neustadt, study no. BAS30. BASF Study no 70 843. BASF DocID 2001/1007645.

Previous evaluation:	Included in Addendum II to DAR (July 2002) for original a.s. approval
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Methods

The effect of mecoprop-P on the seedling emergence of terrestrial plants was studied according to OECD TG 208 (draft July 1999) using BAS 037 32 H containing 600 g/l mecoprop-P.

The study was performed under greenhouse conditions using 6 plant species cultivated in pots. For each plant species, 6 exposure concentrations and a control was tested in 4 replicates performing a pre-emergence application with a spray system. The applied concentrations were BAS 037 32 H at 0, 47, 94, 188, 375, 750 and 1500 ml/ha equivalent to the nominal concentration 0, 28.2, 56.4, 112.8, 225, 450 and 900 g a.s./ha. The test substance was applied pre-emergent dissolved in water equivalent to 200 l/ha. After application the plants were cultivated in the greenhouse for 21 days in plastic pots 7.5 cm diameter containing 180 g soil dry weight. The average temperature was 21.9°C (range 16.8-29.0°C), air humidity average 58.8% (range 27.8-91.7%), and at 16 hour light and 8 hour dark.

The soil medium was steam sterilised natural soil, kaolin, quartz sand and commercial potting soil mixed in the ratio of 1.5 : 0.5 : 1 : 1 v/v.

Table B.9.6.2-09: Characterisation of soil medium

Soil type	PH	Sand (>63 µm)	Silt (2-63 µm)	Clay (<2 µm)	Organic Carbon
Sandy loam	7.6	67.2%	23.1%	9.7%	1.5%

Phytotoxicity (growth reduction and necrosis) were assessed 7, 14 and 21 days after application and estimated in % of affected plants compared to the control.

Results

BAS 037 32 H had no effects on oats. No or only slight to moderate effects were observed for flax, pea and onion. Detrimental effects were observed for oilseed rape and poppy. Most affected was plant weight and plant height of these plants. Visual symptoms and reduction of seedling emergence was not so pronounced. The effects are summarised in the tables below.

Table B.9.6.2-10: Summary of effects relative to control (%)

Plant family	Species	Common name	BAS 037 32 H (ml/ha)	0	47	94	188	375	750	1500
			Nominal concentration (g a.s./ha)	0	28.2	56.4	112.8	225	450	900
Linaceae	<i>Linum usitatissimum</i>	Flax	Emergence	100	107	100	122	111	56	107
			Phytotox	0	0	0	0	0	17	30
			Height	100	94	97	94	86	60	72
			Weight	100	77	92	70	78	45	60
Brassicaceae	<i>Brassica napus</i>	Oilseed rape	Emergence	100	53	92	113	74	92	79
			Phytotox	0	11	10	21	38	70	53
			Height	100	85	76	77	71	27	29
			Weight	100	84	80	72	72	17	14
Leguminosae	<i>Pisum sativum</i>	Pea	Emergence	100	100	87	66	74	92	100
			Phytotox	0	0	0	5	0	5	13
			Height	100	100	102	100	90	94	81
			Weight	100	90	101	100	85	81	62
Papaveraceae	<i>Papaver somniferum</i>	Poppy	Emergence	100	83	108	100	58	63	13
			Phytotox	0	0	0	8	0	0	8
			Height	100	93	100	73	80	58	38
			Weight	100	86	91	73	50	41	23
Poaceae	<i>Avena sativa</i>	Oats	Emergence	100	108	100	108	105	108	100
			Phytotox	0	0	0	0	0	0	0
			Height	100	100	107	106	102	104	98
			Weight	100	95	101	103	99	99	87
Liliaceae	<i>Allium cepa</i>	Onion	Emergence	100	90	125	90	115	110	100
			Phytotox	0	0	0	0	0	8	11
			Height	100	97	92	89	75	74	73
			Weight	100	89	83	89	61	61	61

Table B.9.6.2-11: NOECs, EC25 and EC50 (ml/ha of BAS 037 32 H), 21 days after pre-emergence application

	Flax	Oilseed rape	Pea	Poppy	Oats	Onion
Seedling emergence						
EC ₅₀	>1500	>1500	>1500	864.4	>1500	>1500
EC ₂₅	375<EC ₂₅ <750	>1500	>1500	628.8	>1500	>1500
NOEC	375	1500	1500	188	1500	1500
Phytotoxicity (visual damage)						
NOEC	375	0	375	1500	1500	375
Plant height (shoots above ground)						
EC ₅₀	>1500	522.0	>1500	994.2	>1500	>1500
EC ₂₅	375<EC ₂₅ <750	218.2	>1500	390	>1500	188<EC ₂₅ <750
NOEC	375	188	1500	375	1500	188
Plant weight (shoots above ground)						
EC ₅₀	>1500	443.4	>1500	463.8	>1500	>1500
EC ₂₅	94<EC ₂₅ <188	242.9	750<EC ₂₅ <1500	156	>1500	188<EC ₂₅ <750
NOEC	94	94	750	94	1500	188

Comments

Besides the results as ml/ha of the used test substance, the equivalent amount of mecoprop-P could have been mentioned. RMS has made this calculation in the table below.

Table B.9.6.2-12: NOEC, EC₂₅ and EC₅₀ (equivalent g/ha of MCP-P) 21 day after pre-emergence application

	Flax	Oilseed rape	Pea	Poppy	Oats	Onion
Seedling emergence						
EC ₅₀	>900	>900	>900	518.6	>900	>900
EC ₂₅	225<EC ₂₅ <450	>900	>900	377.2	>900	>900
NOEC	225	900	900	112.8	900	900
Phytotoxicity (visual damage)						
NOEC	225	0	225	900	900	225
Plant height (shoots above ground)						
EC ₅₀	>900	313.2	>900	596.5	>900	>900
EC ₂₅	225<EC ₂₅ <450	130.9	>900	234	>900	112.8<EC ₂₅ <450
NOEC	225	112.8	900	225	900	112.8
Plant weight (shoots above ground)						
EC ₅₀	>900	266	>900	278.3	>900	>900
EC ₂₅	56.4<EC ₂₅ <112.8	145.7	450<EC ₂₅ <900	93.6	>900	112.8<EC ₂₅ <450
NOEC	56.4	56.4	450	56.4	900	112.8

The lowest EC₅₀ on seedling emergence was poppy with 518.6 g a.s./ha.

The lowest EC₅₀ for plant height and weight of shoots above ground was oilseed rape with EC₅₀ 313.2 and 266 g a.s./ha, respectively.

RMS comments (renewal):

Study not revisited at renewal by RMS. Critical study endpoint confirmed from above summary to be: ER₅₀ = 266 g a.s./ha, for plant weight reduction with oilseed rape

Reference

Frank P (2001b): BAS 037 32 H. A toxicity test to determine the effects of the test item on vegetative vigour of terrestrial plants. Staatliche Lehr- und Forschungsanstalt für Landwirtschaft (SLFA), Neustadt, study no. BAS33. BASF Study no 70 845. BASF DocID 2001/1007646.

Previous evaluation:	Included in Addendum II to DAR (July 2002) for original a.s. approval
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Methods

The effect of mecoprop-P on the vegetative vigour of terrestrial plants was studied according to OECD TG 208 (draft July 1999) using BAS 037 32 H containing 600g/l mecoprop-P (measured 596.1 g/l). The study resembles the above mentioned with the exception that application takes place after emergence.

The study was performed under greenhouse conditions using 6 plant species cultivated in pots. For each plant species, 6-7 exposure concentrations and a control was tested in 4 replicates each of 3-5 plants/pot performing a post-emergence application with a spray system. The applied concentrations were BAS 037 32 H at 0, 265, 375, 530, 750, 1061, 1500, 2121, and 3000 ml/ha equivalent to the nominal concentration 0, 159, 225, 318, 450, 636, 900, 1273 and 1800 g mecoprop-P/ha. The test substance was applied dissolved in water equivalent to 200 l/ha. After application the plants were cultivated in the greenhouse for 21 days in plastic pots 7.5 cm diameter containing 180 g soil dry weight. The average temperature was 21.8°C (range 17.3-27.2°C), air humidity average 66.3% (range 31.1-97.2%), and at 16 hour light and 8 hour dark.

The soil medium was steam sterilised natural soil, kaolin, quartz sand and commercial potting soil mixed in the ratio of 1.5 : 0.5 : 1 : 1 v/v.

Table B.9.6.2-13: Characterisation of soil medium.

Soil type	pH	Sand (>63 µm)	Silt (2-63 µm)	Clay (<2 µm)	Organic Carbon
Sandy loam	7.6	67.2%	23.1%	9.7%	1.5%

Phytotoxicity (growth reduction and necrosis) were assessed 7, 14 and 21 days after application in growth stages 12-14 (BBCH-Code) and estimated in % of affected plants volume compared to the control.

Table B.9.6.2-14: Summary of results in % of control.

Plant family	Species	Common name	BAS 037 32 H (ml/ha)	0	265	375	530	750	1060	1500	2121	3000
			Nominal concentration (g a.s./ha)	0	159	225	318	450	636	900	1273	1800
Linaceae	<i>Linum usitatissimum</i>	Flax	Phytotoxicity	0	-	16	12	32	35	37	53	-
			height	100	-	83	81	71	65	58	52	-
			weight	100	-	76	77	58	44	39	30	-
Brassicaceae	<i>Brassica napus</i>	Oilseed rape	phytotox	0	73	73	88	95	90	90	-	-
			height	100	68	64	62	51	51	49	-	-
			weight	100	19	15	11	3	7	5	-	-
Leguminosae	<i>Pisum sativum</i>	Pea	phytotox	0	-	-	12	28	33	33	55	70
			height	100	-	-	89	79	77	80	60	45
			weight	100	-	-	73	65	69	71	49	27
Papaveraceae	<i>Papaver somniferum</i>	Poppy	phytotox	0	10	23	47	65	85	85	-	-
			height	100	89	84	68	61	52	45	-	-
			weight	100	69	65	45	38	15	7	-	-
Poaceae	<i>Avena sativa</i>	Oats	phytotox	0	-	-	0	0	0	0	0	0
			height	100	-	-	101	100	99	97	93	92
			weight	100	-	-	98	95	93	88	80	76
Liliaceae	<i>Allium cepa</i>	Onion	phytotox	0	-	-	0	1	12	14	23	30
			height	100	-	-	92	93	88	89	86	83
			weight	100	-	-	92	90	76	66	58	38

:- Concentration not included.

Results

BAS 037 32 H had only minor adverse effects to oats. Application up to 3 l/ha BAS 037 32 H may cause damages to oilseed rape, flax, pea, poppy and onion. The following order of sensitivity among the tested plants were observed: Oilseed rape > poppy > flax > pea > onion > oats.

Table B.9.6.2-15: NOEC, EC₂₅ and EC₅₀ (ml/ha of BAS 037 32 H), 21 days after post-emergence application.

	Flax	Oilseed rape	Pea	Poppy	Oats	Onion
Phytotoxicity (visual damage)						
NOEC	0	0	0	265	3000	750
Plant height (shoots above ground)						
EC ₅₀	2201	1233	2751	1193	>3000	>3000
EC ₂₅	639	<265	1417	454	>3000	>3000
NOEC	0	0	1500	265	1500	750
Plant weight (shoots above ground)						
EC ₅₀	1002	<265	1940	490	>3000	2345
EC ₂₅	439	<265	823	260	>3000	1210
NOEC	530	0	0	0	1500	750

Comments

Besides the results as ml/ha of the used test substance the equivalent amount of mecoprop-P could have been mentioned. RMS has made this calculation in the table below.

Table B.9.6.2-16: NOEC, EC₂₅ and EC₅₀ (equivalent g/ha of MCP-P), 21 days after post-emergence application.

	Flax	Oilseed rape	Pea	Poppy	Oats	Onion
Phytotoxicity (visual damage)						
NOEC	0	0	0	159	1800	450
Plant height (shoots above ground)						
EC ₅₀	1320	740	1651	716	>1800	>1800
EC ₂₅	383	<159	850	272	>1800	>1800
NOEC	0	0	900	159	1800	450
Plant weight (shoots above ground)						
EC ₅₀	601	<159	991	294	>1800	1407
EC ₂₅	263	<159	494	156	>1800	726
NOEC	318	0	0	0	1800	450

The lowest EC₅₀ for plant height and weight of shoots above ground was poppy with EC₅₀ = 716 and oilseed rape with EC₅₀ <159 g a.s./ha, respectively.

The NOEC cannot be derived from the results. Presenting a NOEC 0 is correct of course (no exposure) but of no use in this context. It could be presented as below the lowest applied concentration which will also be misleading with e.g. 73% damage in oilseed rape at the lowest used application rate.

RMS comments (renewal):

Study not revisited at renewal by RMS. Critical study endpoint confirmed from above summary to be: ER₅₀ = 294 g a.s./ha, for plant weight reduction with Poppy.

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

No additional studies submitted for the purpose of renewal.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

Ref.: IIA. 8.7. Hamm, 1987: Influence of mecoprop on the growth of *Pseudomonas putida*.

Previous evaluation:	Included in DAR (1998) for original a.s. approval
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Methods

The effect of mecoprop (99.55% pure) on micro-organisms was studied by performing a *Pseudomonas putida* cell reproduction inhibition test according to the guideline DIN 38412, part 8.

The test concentrations were 0, 62.5, 125, 250, 500, 1000 and 2000 mg/l dissolved in distilled water containing 0.01% Tween 80 with 6 batches per concentration. The test batches were placed in a shaking incubator and incubated for 17 hours at 21°C. The inhibition on bacterial growth was determined by calculations based on the cell densities measured by photometer.

Results

The EC₅₀ (17 hours) was 170 mg/l based on graphic determination.

The EC₁₀ (17 hours) was 20 mg/l and NOEC (17 hours) was 12 mg/l.

Table B.9.8-01: Bacterial Growth inhibition

	The inhibition on bacterial growth at the concentration (mg MCPP/l):						
	0	62.5	125	250	500	1000	2000
Inhibition %	0.0	32.0	45.8	50.9	67.2	85.2	95.3

RMS Comments

A study intending to clarify the effects on sewage treatment should have been based on activated sludge from a waste water treatment plant and performed according to available guidelines. Tests on individual bacterial populations must be considered less relevant. However, the effects on *Pseudomonas putida* may be used in this assessment.

RMS comments (renewal):

The study has not been revisited for the purposes of active substance renewal. The EC₅₀ is confirmed from the original RMS evaluation to be 170 mg a.s./L

Report:	CA 8.8/01, Falk, S (2013)
Title	Toxicity to microorganisms activated sludge respiration inhibition test Report No. S13-00244
Guidelines:	OECD 209
GLP:	Yes
Deviations	<ul style="list-style-type: none"> - the pH was below 7.0 during the test - the number of replicates was deviant from the protocol

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS**A. MATERIALS**

- Test materials:** Mecoprop-P
Description: Light yellow solid
Lot/Batch #: 3860
Purity: 92.63 % w/w
CAS #: 16484-77-8
Stability of test compound: Stable
Reference item: 3,5-dichlorophenol
- Test species:** Aerobic activated sludge (micro-organisms from domestic wastewater treatment plant) supplied by the sewage works of Pforzheim, Germany.
Test item loading rate: 10, 32, 100, 320 and 1000 mg Mecoprop-P/L
Feeding: Synthetic waste water; recipe according to OECD 209.

Duration:	3 hours
Temperature:	18.2 – 20.7°C
pH:	6.01 – 7.72

B. STUDY DESIGN AND METHODS

1. Dates of work:

20 February – 06 March 2013

2. Experimental conditions

The microbial inoculum was activated sludge collected one day before the study start from a sewage treatment works in Pforzheim, Germany that treats predominantly domestic sewage. The activated sludge was washed three times with chlorine free tap water and between washings was centrifuged for 10 minutes at 1000 rpm. During storage, 50 mL synthetic sewage feed was added per litre activated sludge each day. The mixed liquor suspended solids (MLSS) were adjusted to a concentration of 3.0 g/L ($\pm 10\%$). The activated sludge was stirred and aerated during storage.

The test was performed using synthetic waste water. The solution was sterilised prior to storage.

Test mixtures containing water, synthetic waste water and the test items were prepared and dispersed in the flasks (glass 500 mL volume) by intense aeration. There were 6 control replicates prepared, 3 replicates per concentration of test item, and a single replicate per reference item concentration. An abiotic control was prepared with the highest concentration of test item, water and synthetic waste water. An additional set of replicates was prepared to assess nitrification-based oxygen consumption. These were performed for each concentration of the test assay and for the reference assay. Nitrification replicates were prepared using additionally 2.5 mL of ATU solution (2.32 g/L).

The test was started by adding activated sludge in solution (250 mL per replicate) to the treated water and waste water mixes (250 mL per replicate). Measurements of the oxygen uptake of the activated sludge were performed 3 hours after starting the respiration.

3. Dose selection

A range finding test was performed using concentrations of 10, 100 and 1000 mg/L. Since statistically significant effects were observed at 1000 mg/L the main test was performed using concentrations of 10, 32, 100, 320 and 1000 mg Mecoprop-P/L. The reference item 3,5-Dichlorophenol was tested at concentrations of 1, 3, 9 and 25 mg/L.

4. Statistics

For the respiration rate of the test item and control, SAS® (2002-2008) service pack 9.2 was used. A test for normality of the data was performed using Shapiro-Wilk's statistic. A test for homogeneity of variance for the data was performed using the Levene test. The NOEC was determined using Dunnett's. EC₅₀s were calculated using probit analysis following the Gompertz distribution.

II. RESULTS AND DISCUSSION

A. FINDINGS

All guideline validity criteria were fulfilled according to OECD 209 as follows:

- Control total respiration was at least 20 mg O₂/g/hr = mean of 22.5 mg/g/hr.
- The Coefficient of Variation for control replicate total respiration was ≤ 30% = 9.5%
- The EC₅₀ for 3,5-Dichlorophenol inhibition of total respiration was in the range 2-25 mg/L = calculated as 3-9 mg/L

Respiration rates in the control and test item treatments after 3 hours exposure are summarised in Table B.9.8-02.

Table B.9.8-02: Respiration rates in the controls and test item treated vessels

Test assay nominal (mg/L)	Mean respiration (mg O ₂ /g/hr.) [% inhibition vs. control]		
	Total	Heterotrophic	Nitrification
Control	22.48	13.58	8.91
10.0	21.94 [2.4]	13.90 [-2.4]	8.04 [9.7]
32.0	22.26[1.0]	14.97 [-10.2]	7.29 [18.1]
100	21.27[5.4]	13.36 [1.6]	7.91[11.1]
320	16.79 [25.3]*	13.36[1.6]	3.32 [61.5]*
1000	8.93 [60.3]*	6.71[50.6]*	2.22 [75.1]*
DCP 1	17.51 [22.1]	18.92[-39.3] [†]	-1.41 [115.8] [†]
DCP 3	15.59 [30.6]	13.58 [0.0]	2.01 [77.4]
DCP 9	8.94 [60.2]	8.55 [37.0]	0.39 [95.6]
DCP 25	2.69 [88.0]	2.97 [78.1]	-0.28 [103.1]

* statistically significant compared to the control

[†] Excluded from the calculation due to measuring errors

[values] – inhibition versus control group

III. CONCLUSIONS

After 3 hours, statistically significant effects were observed for the total oxygen uptake at 320 and 1000 mg/L for the total oxygen uptake. The NOEC was 100 mg/L and the LOEC was 320 mg/L. The EC₅₀ was determined to be 767 mg/L.

For the heterotrophic oxygen uptake, after 3 hours, statistically significant effects were observed at 1000 mg/L. The NOEC was 320 mg/L and the LOEC was 1000 mg/L. The EC₅₀ was determined to be >1000 mg/L.

For the oxygen uptake due to nitrification, statistically significant effects were observed at 320 and 1000 mg/L. The NOEC was 100 mg/L and the LOEC was 320 mg/L. The EC₅₀ was determined to be 319 mg/L.

RMS comments:

The study was well reported and conducted in adherence with OECD guideline 209, with all related validity criteria met. There were some minor methodology deviations in the study, such as the measured pH in some replicates falling to below the minimum of 7.0 recommended in the guideline. However all replicates were within the range 7-8 at test initiation and the trend of results indicate that this decrease was a likely impact of the test item, as pH values were generally lower at higher tested concentrations of mecoprop-P. This deviation is not considered to adversely impact the reliability of the study. Overall the study is considered to be valid and acceptable for risk assessment purposes. The agreed endpoints are as follows:

- NOEC = 100 mg a.s./L, based on inhibition of total respiration.

- $EC_{50} = 319$ mg a.s./L, based on inhibition of nitrification respiration.

B.9.9. MONITORING DATA

Please refer to Volume 3 (CA) B.8.4 for environmental monitoring data.

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

The groundwater fate modelling in the Environmental fate section B.8.3 has identified no metabolites that occur in groundwater. As such no further consideration is required.

B.9.11. REFERENCES RELIED ON (INCLUDING LITERATURE REVIEW)

B.9.11.1. Literature review

Background

A literature review has been carried out active substance mecoprop-p (CAS 16484-77-8) and synonyms, along with its 2-ethyl hexyl ester (CAS 861229-15-4), and its potassium (CAS 66423-05-0) and dimethylamine (DMA) salts (CAS 66423-09-4). Details of this literature search are summarised below.

The review has been conducted by the Notifier in accordance with requirements under Article 8(5) of Regulation (EC) No. 1107/2009, based on EFSA guidance as published in EFSA Journal 2011; 9(2):2092.

The objective of the review is to determine if any scientific peer-reviewed open literature, published in the last 10 years before the submission date of the dossier (i.e. 2004 – 2014), is suitable for consideration in the risk assessment for mecoprop-p.

The RMS has evaluated the quality of the conducted literature review in relation to its ability to identify relevant and reliable published information suitable for consideration in the ecotoxicology risk assessment.

i) Evaluation of comprehensiveness of information databases used in the search

A total of 26 bibliographic databases were used in the conducted search, covering both human health and environmental aspects of the regulatory evaluation. The Notifier's justification for the use of these databases is that:

“Proquest Dialog is a premier online retrieval service with a comprehensive database collection and powerful search language.”

A complete list of the databases included in the search is provided below:

- AGRICOLA Professional
- AGRIS
- Aquatic Science and Fisheries Abstracts (ASFA)
- BIOSIS Previews®
- British Library Inside Conferences
- CAB Abstracts
- Chemical Safety NewsBase
- Current Contents Search®
- Embase®
- Embase® Alert
- Foodline®: SCIENCE

-
- HSELINE: Health and Safety
 - Incidence & Prevalence Database
 - International Pharmaceutical Abstracts
 - Lancet Titles
 - MEDLINE®
 - New England Journal of Medicine
 - Oceanic Abstracts
 - PASCAL
 - Pollution Abstracts
 - ProQuest Biological & Health Science Professional
 - ProQuest Environmental Science Professional
 - Registry of Toxic Effects of Chemical Substances (RTECS®)
 - SciSearch®: a Cited Reference Science Database
 - Toxfile®
 - Water Resources Abstracts

With respect to identifying ecotoxicology related published literature, the RMS considers that the number and range of different databases used was quite extensive and that there are therefore no significant deficiencies identified with regards to this aspect of the conducted literature review.

ii) Evaluation of database search strategy:

All searches were undertaken using the ‘single concept’ strategy as described by the relevant EFSA guidance document (EFSA Journal 2011;9(2):2092).

a) Search strategy for the active substance (includes human health and environment):

Within the ‘ProQuest’ Dialogue database collection, the following search terms were utilised in an attempt to capture literature referring to the active substance mecoprop-p, or any of its known synonyms:

Mecoprop-p **OR** 16484-77-8 **OR** (+)-Mcpp2 **OR** (R)-2-(4-Chloro-2-Methylphenoxy)propanoic acid **OR** (R)-Mecoprop **OR** 2M-4XP **OR** Duplosan KV **OR** 240-539-0 **OR** Mecoprop, D- **OR** d-Mecoprop **OR** (R)-2-(4-Chloro-2-methylphenoxy)propionic acid **OR** Propanoic acid, 2-(4-chloro-2-methylphenoxy)-, (2R)- **OR** Propanoic acid, 2-(4-chloro-2-methylphenoxy)-, (R)- (9CI) **OR** Propionic acid, 2-((4-chloro-o-tolyl)oxy)-, (+)- **OR** (R)-2-(4-Chloro-o-tolyloxy)-propionic acid **OR** MCPP-p **OR** CMPP-p **OR** (Optica **AND** Mecoprop-p)

It was considered by the RMS that as mecoprop-P and MCPP-P are the common names for this active substance, and so it would be expected that any relevant article from the search period would make reference to this term somewhere within the text of the article. The supplementary search terms used, including the chemical (IUPAC) name and CAS /Registry number (as suggested in the EFSA guidance) give increased confidence that all relevant articles would be captured.

b) Search strategy for metabolites (includes human health and environmental):

The following search terms were used in order to capture any literature articles referring to the metabolites of mecoprop-P; 2-Ethyl Hexyl Ester (2-EHE), the potassium salt and the dimethylamine (DMA) salt :

- (2-ethyl hexyl ester **AND** (Mecoprop-p and Synonyms)) **OR** 861229-15-4
- Potassium **AND** (Mecoprop-p and Synonyms)) **OR** 66423-05-0 **OR** Zolaprofos

- Dimethylamine salt **AND** (Mecoprop-p and Synonyms)) **OR** 66423-09-4

It was considered by the RMS that the search for each metabolite using both the common name in conjunction with either the chemical CAS number or the known names for the parent active substance would direct the search appropriately, without being overly restrictive. The searched metabolites, however, do not include o-cresol, which was potentially relevant for the surface water risk assessment.

c) ‘Limitations’ included in active substance and metabolite searches:

The searches were limited to literature published since 2004. This is in line with Article 8(5) of Regulation (EC) No 1107/2009 which states that applicants should include in the dossier the most recent scientific peer-reviewed open literature published during ten years prior to the dossier submission date. Also, the results were filtered to limit the output to non-patent documents.

There was an additional limitation placed on the active substance search to aim to ensure relevance to the active substance in question. An initial search for the tradename ‘Optica’ returned 200,000 articles. It was proposed that ‘Optica’ was likely to be a tradename for several substances or items and so many hits would likely have no relevance to mecoprop-p itself. As such the limitation that ‘Optica’ would also need to include ‘Mecoprop-p’ was added to the search for references. The same practise was undertaken for the search term ‘mcpp’

d) RMS’s evaluation of quality of search strategy:

With respect to ecotoxicology related requirements, the RMS is in agreement with the active substance and metabolite search strategies. The included ‘limitations’ are largely agreed with. However it could be considered slightly restrictive to only search for the terms ‘Optica’ and ‘mcpp’ in conjunction with the single term ‘Mecoprop-P’. A more thorough approach might have been to search for these ‘high hit’ terms alongside each known synonym of the active substance. It is appreciated that this would greatly increase the complexity of the literature search and overall the RMS believes that the majority, if not all relevant articles would still be likely to have been captured using the applicant approach.

It is concluded with respect to ecotoxicology aspects, that the conducted ‘single concept’ search strategy and associated limitations adequately meets the ‘literature search’ related requirements in this area, with no significant deficiencies.

iii) Evaluation of ‘relevance’ of publications

The Notifier’s initial assessment of relevance was conducted based on the publication’s title only and related to all data requirement areas (i.e. covering human health and environmental aspects). Each article title was manually reviewed and for any relevant or potentially relevant records abstracts were obtained so that a more thorough consideration could be made. This first step approach is broadly in line with the ‘rapid assessment’ described within the EFSA guidance document on conducting literature searches (2011), under which *a summary record may be excluded on the basis of the title alone (e.g. if an abstract is not available), provided that the title provides sufficient information to clearly indicate non-relevance.*

In the second step, the abstracts were obtained for the remaining papers and a second selection was made. The papers which clearly did not meet the criteria were rejected. As a third step, the full-length papers were examined in order to ascertain their eligibility (including reliability, transparency and repeatability) and collect data.

Step 1 relevance evaluation for active substance and its metabolites (all data requirement areas):

The initial literature search as described above for the active substance; Mecoprop-P returned a total of 117 ‘hits’. The related search for the 3 metabolites returned a total of 3 further articles: none for 2-ethyl hexyl ester, 1 for the potassium salt and 2 for the dimethylamine (DMA) salt. After implementation of the notifier’s step 1 relevance check (title only) this number was reduced to 36

titles. The 2 hits for the dimethylamine salt metabolite were found to have also been found in the active substance search and so were not reconsidered. For the single ‘hit’ for the potassium salt full details were retrieved. As this proved to be a suggested journal title, no abstracts were retrieved.

Step 2 relevance evaluation for the active substance and metabolites (Ecotoxicology related areas):

The identified 36 ‘Step 1 relevant’ or ‘of unclear relevance’ active substance articles were reduced to a remainder of 12 articles following the step 2 detailed consideration of abstracts. This list of sifted articles is provided in table 9.11.1-01 below:

Table CA 9.11.1-01 : Report of all relevant studies and studies of unclear relevance (ordered by author)

Author(s)	Data requirements	Year	Title	Source
Beinum, W van , Beulke, S , Sinclair, C J , Smart, R , Brown, C D	IIA 7.4.7	2007	The effect of soil type on pesticide leaching.	Environmental fate and ecological effects of pesticides
Buss , Thrasher, J , Morgan, P , Smith, JWN	IIA 7.3.1.1	2006	A review of Mecoprop attenuation in the subsurface	QUARTERLY JOURNAL OF ENGINEERING GEOLOGY AND HYDROGEOLOGY
Degenhardt, Dani , Cessna, Allan J. , Raina, Renata , Farenhorst, Annemieke , Pennock, Dan J.	IIA 7.8.3	2011	DISSIPATION OF SIX ACID HERBICIDES IN WATER AND SEDIMENT OF TWO CANADIAN PRAIRIE WETLANDS	Environmental Toxicology and Chemistry
Idowu, I A , Alkhaddar, R M , Atherton, W	IIA 7.4.7	2014	Possible source term of high concentrations of Mecoprop-p in leachate and water quality: impact of climate change, public use and disposal.	Environmental Technology
Loos, Robert , Locoro, Giovanni , Contini, Serafino	IIA 7.12	2010	Occurrence of polar organic contaminants in the dissolved water phase of the Danube River and its major tributaries using SPE-LC-MS2 analysis	WATER RESEARCH
Mottier, A , Kientz-Bouchart, V , Dubreule, C , Serpentin, A , Lebel, J M , Costil, K	IIA 8.3.1.4	2014	Effects of acute exposures to Mecoprop, Mecoprop-p and their biodegradation product (2-MCP) on the larval stages of the Pacific oyster,	Aquatic Toxicology

Author(s)	Data requirements	Year	Title	Source
			Crassostrea gigas .	
Nestorovska-Krsteska, Aleksandra , Mirceska, Meri , Aaron, Jean-Jacques , Zdravkovski, Zoran	IIA 7.12	2008	Determination of dimethoate, 2,4-dichlorophenoxy acetic acid, Mecoprop and linuron pesticides in environmental waters in republic of Macedonia by high performance liquid chromatography	MACEDONIAN JOURNAL OF CHEMISTRY AND CHEMICAL ENGINEERING
Nolan, B T , Dubus, I G , Surdyk, N , Gautier, A , Crouzet, C , Flehoc, C	IIA 7.4.1	2007	Sorption of 7 weak-acid pesticides in 41 European soils: controlling factors and empirical modelling.	Environmental fate and ecological effects of pesticides
Piwowarczyk, Agnieszka A. , Holden, Nicholas M.	IIA 7.4.1	2013	Phenoxyalkanoic acid herbicide sorption and the effect of co-application in a Haplic Cambisol with contrasting management	Chemosphere
Rice, P J , Horgan, B P , Rittenhouse, J L	IIA 7.12	2010	Pesticide transport with runoff from creeping bentgrass turf: relationship of pesticide properties to mass transport.	Environmental Toxicology and Chemistry
Rodriguez-Cruz MS, Baelum, Jacob , Shaw, Liz J , Sorensen, Sebastian R , Shi, Shengjing , Aspray, Thomas , Jacobsen, Carsten S , Bending, Gary D	IIA 7.3.1.1	2010	Biodegradation of the herbicide Mecoprop-p with soil depth and its relationship with class III tfdA genes	Soil biology & biochemistry.
Zhao, Y Q , Singleton, P , Meredith, S , Rennick, G W	IIA 7.12	2013	Current status of pesticides application and their residue in the water environment in Ireland.	International Journal of Environmental Studies

The following articles were not further considered after step 2 detailed consideration of the associated abstract:

Table CA 9.11.1-02 : Those articles excluded from the risk assessment after detailed assessment of abstract

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier
Broschat, T K , Busey, P	2010	Toxicity of turfgrass postemergence herbicides to <i>Wodyetia bifurcata</i> .	Palms	Efficacy & selectivity - not relevant
Damgaard, C , Mathiassen, S K , Kudsk, P	2008	Modeling effects of herbicide drift on the competitive interactions between weeds.	Environmental Toxicology and Chemistry	Effect of mecoprop-P on competitive interactions of plants = not relevant to any endpoints.
Degenhardt, Dani	2010	Herbicide dynamics in prairie wetlands.	Dissertation Abstracts International. Vol. 74, no. 06, suppl. B, 154 p. 2010.	Canadian data.
Deihimfard, R , Zand, E , Damghani, A M , Soufizadeh, S	2007	Herbicide risk assessment during the Wheat Self-sufficiency Project in Iran.	Pest Management Science	Herbicide use patterns in Iran = not relevant to EU.
Evangelista, S. , Cooper, D. G. , Yargeau, V.	2010	The effect of structure and a secondary carbon source on the microbial degradation of chlorophenoxy acids	CHEMOSPHERE	Microbial degradation of phenoxys. BUT on racemic Mecoprop. Will not have any influence on any endpoints.
Fogg, P , Boxall, A B A , Walker, A , Jukes, A	2004	Effect of different soil textures on leaching potential and degradation of pesticides in biobeds.	Journal of Agricultural and Food Chemistry	Methodology of removal of chemicals from water - not relevant
Glozier, Nancy E , Struger, John , Cessna, Allan J , Gledhill, Melissa , Rondeau, Myriam , Ernst, William , Sekela, Mark A , Cagampan, Steve J , Sverko, Ed , Murphy, Clair , Murray, Janine L , Donald, David B	2012	Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007	Environmental Science and Pollution Research International	Monitoring data from water in Canada. Not relevant for EU.
Glozier, Nancy E , Struger, John , Cessna, Allan J , Gledhill, Melissa , Rondeau, Myriam , Ernst, William R , Sekela, Mark A , Cagampan, Steve J , Sverko, Ed , Murphy, Clair , Murray, Janine L , Donald, David B	2011	Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007	Environmental science and pollution research international	REPEAT ENTRY
Gouze, M. , Klegou, G. , Fastier, A.	2011	Setting dermal absorption values for active substances in pesticide formulations:	Toxicology Letters (Shannon)	

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier
		A read-across approach		
Grobela, M , Grzesiak, P , Motaa, R	2011	Heavy metal complexes with dicamba, Mecoprop-p and MCPB.	Progress in Plant Protection	Mode of action - not relevant
Hatterman-Valenti, H , Mayland, P	2005	Annual flower injury from sublethal rates of dicamba, 2,4-D, and premixed 2,4-D+Mecoprop+dicamba.	HortScience	Only used a mixture product containing Mecoprop and only discusses efficacy = not relevant.
Huntscha, Sebastian , Velosa, Diana M. Rodriguez , Schroth, Martin H. , Hollender, Juliane	2013	Degradation of Polar Organic Micropollutants during Riverbank Filtration: Complementary Results from Spatiotemporal Sampling and Push-Pull Tests	Environmental Science & Technology	Riverbank filtration not relevant.
Kamp, H. G. , Fabian, E. , Herold, M. , Krennrich, G. , Leibold, E. , Looser, R. , Mellert, W. , Prokoudine, A. , Strauss, V. , Walk, T. , Wiemer, J. , van Ravenzwaay, B.	2009	Metabolite profiling, a tool for early detection of hepatotoxins and their toxicological mechanisms in rats	Naunyn-Schmiedeberg's Archives of Pharmacology	
Kopmanis, J	2005	Changes in susceptibility of next generation of common lambsquarter (<i>Chenopodium album</i> L.) to applied herbicides	Latvijas Lauksaimniecības universitāte. Raksti (Latvia); Proceedings of the Latvia University of Agriculture	Efficacy - not relevant
Kurt-Karakus, P B , Bidleman, T F , Muir, D C G , Cagampan, S J , Struger, J , Sverko, E , Small, J M , Jantunen, L M	2008	Chiral current-use herbicides in Ontario streams.	Environmental Science & Technology	Monitoring data from water in Canada. Not relevant for EU.
Kurt-Karakus, P B , Bidleman, T F , Muir, D C G , Struger, J , Sverko, E , Cagampan, S J , Small, J M , Jantunen, L M	2010	Comparison of concentrations and stereoisomer ratios of Mecoprop, dichlorprop and metolachlor in Ontario streams, 2006-2007 vs. 2003-2004.	Environmental Pollution	Monitoring data from water in Canada. Not relevant for EU.
Kurt-Karakus, Perihan Binnur , Bidleman, Terry F , Muir, Derek C G , Struger, John , Sverko, Ed , Cagampan, Steve J , Small, Jeff M , Jantunen, Liisa M	2009	Comparison of concentrations and stereoisomer ratios of Mecoprop, dichlorprop and metolachlor in Ontario streams, 2006-2007 vs. 2003-2004	Environmental pollution (Barking, Essex : 1987)	REPEAT ENTRY

Author(s)	Year	Title	Source	Reason(s) for not including this study in the dossier
Mottier, Antoine , Kientz-Bouchart, Valérie , Dubreule, Christelle , Serpentine, Antoine , Lebel, Jean Marc , Costil, Katherine	2013	Effects of acute exposures to Mecoprop, Mecoprop-p and their biodegradation product (2-MCP) on the larval stages of the Pacific oyster, <i>Crassostrea gigas</i>	Aquatic toxicology (Amsterdam, Netherlands)	REPEAT ENTRY
Müller, Tina A , Zavodszky, Maria I , Feig, Michael , Kuhn, Leslie A , Hausinger, Robert P	2006	Structural basis for the enantiospecificities of R- and S-specific phenoxypropionate/alpha-ketoglutarate dioxygenases	Protein science : a publication of the Protein Society	Mode of action - not relevant
Rice, P J , Horgan, B P , Rittenhouse, J L	2010	Evaluation of core cultivation practices to reduce ecological risk of pesticides in runoff from <i>Agrostis palustris</i> .	Environmental Toxicology and Chemistry	Comparison of cultivation practices to reduce concentrations = not relevant to any endpoints.
Santilio, A , Stefanelli, P , Girolimetti, S , Dommarco, R	2011	Determination of acidic herbicides in cereals by QuEChERS extraction and LC/MS/MS.: Special Issue: Pesticides in the Mediterranean area.		Not relevant - analytical methods
(Anon.)	2013	Reasoned opinion on the review of the existing maximum residue levels (MRLs) for Mecoprop and Mecoprop-p according to Article 12 of Regulation (EC) No 396/2005.		MRL document publically available - no need to summarise
(Anon.)	2007	Propionic acid, 2-((4-chloro-o-tolyl)oxy)-		
	2010	Effect of chemical plant protection on grain quality of spring wheat.		Not relevant - efficacy

With regards to those studies considered relevant and reports obtained for consideration the RMS agrees with the notifier justifications for all 12 articles/studies. The justifications provided by the notifier are as follows:

- Beinum, W van et al (2007): Lysimeter studies to study effect of soil type on mecoprop-P leaching.
- Buss et al (2006): Discusses UK groundwater levels.
- Degenhardt, D et al (2011): Dissipation of mecoprop-P in a water/sediment system.
- Idowu I A et al (2014): Discusses mecoprop-p in groundwater and surface water in the UK
- Loos, R. et al (2010): Monitoring data for River Danube.
- Mottier, A. et al (2014): Would not normally consider oysters as a species, but the report does say that pesticides are sometimes detected at high levels in seawater. This study is not harmful as the results show toxicity levels are low.
- Nestorovka-Krsterka, A. et al (2008): Pesticide concentrations in Macedonia.

- Nolan, B T. et al (2007): Sorption studies on mecoprop-P.
- Piwowarczyk, A. et al (2013): Information on the sorption of mecoprop-P and MCPA.
- Rice, P J. et al (2010): Mobility of mecoprop-P discussed.
- Rodriguez-Cruz, M S et al (2010): Unclear relevance. Title: *Biodegradation of the herbicide Mecoprop-p with soil depth and its relationship with class III tfdA genes*
- Zhao, Y Q et al (2013): Discusses residue in Irish water.

Those studies/articles excluded at step 3 are documented in the above table CA 9.11.1-02. Varying reasons are stated by the notifier. For example, the *Reasoned opinion on the review of the existing maximum residue levels (MRLs) for Mecoprop and Mecoprop-p...* is a publicly available document and so will not offer any additional endpoints over those already considered in the renewal assessment. Other articles/studies are clearly not relevant to the environmental aspect of the risk assessment, or were repeat entries from multiple publications.

Overall the RMS has checked the provided abstracts for these excluded studies and is in agreement with the criteria applied by the notifier.

Of the 12 studies/articles judged as relevant for informing the environmental risk assessment are stated as evaluated by the notifier for reliability, repeatability and transparency aspects. Full submitted study evaluation summaries are provided into sections B.8 and B.9 (AS) as appropriate and are considered in detail by the RMS within the relevant section. It should be noted that, of the 12 literature studies deemed as relevant to inform the environmental assessment, only one of these is relevant to the ecotoxicology of Mecoprop-P. All others are more directly related with the fate and behaviour of the active substance/metabolites and so will be considered in section B.8 (AS) of the Renewal Assessment Report. The sole study relevant for ecotoxicological consideration is as follows:

Table CA 9.11.1-03 : Literature study deemed relevant for informing the ecotoxicological assessment of mecoprop-p

OECD point	Study number	Author(s)	Test Substance	Organism	Study endpoint(s)	Notifier comments
IIA 8.3.1.4	KCA 8-01	Mottier <i>et al.</i> , 2014	Mecoprop Mecoprop-P 2-MCP	Organisms : Larvae of Pacific oyster, <i>Crassostrea gigas</i>	EC ₁₀ and EC ₅₀ determined on measured concentrations in µg/L (unless for 2-MCP for which the measured concentration could not be determined)	Endpoints obtained on oyster larvae could be added at the EFSA list of ecotoxicity endpoint of mecoprop and mecoprop-P. Endpoints determined on oyster larvae do not change the conclusions on ecotoxicity of mecoprop on aquatic organisms since this compound is known to be slightly toxic to aquatic organisms.

Evaluation and summary of the above study by the RMS is provided below:

Report:	CA 8/01, Mottier, A. <i>et al.</i> , 2014
Title	Effects of acute exposures to mecoprop, mecoprop-p and their biodegradation product (2-MCP) on the larval stages of the Pacific oyster, <i>Crassostrea gigas</i>
Guidelines:	Not stated
GLP:	Not stated
Deviations	Not applicable

Previous evaluation:	None: Submitted for the purpose of renewal under Regulation 844/2012
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I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

Mecoprop [2(4-chloro-2-methylphenoxy) propanoic acid]
Chemical name C₁₀H₁₁ClO₃
Chemical formula 7085-19-0
CAS Number 99.5%
Purity Mixture of two stereo isomers in an equal proportion
Description

Mecoprop-P

CAS Number 16484-77-8
Purity 99%
Description Formulation containing the (R)-(+)-enantiomer

2-MCP

Chemical name 2-Methyl-4-chlorophenol
Chemical formula C₇H₇ClO
CAS Number 1570-64-5
Purity 99%
Description Degradation product form Mecoprop; Mecoprop-P and other pesticides

2. **Vehicle and /or positive controls** Sea water and CuSO₄.5H₂O

3. Test animals-

Species: Crassostrea gigas
Age: Embryotoxicity assay: Veliger larvae (also called D-shaped larvae in the paper)
Metamorphosis assay: Pediveliger larvae (21 days old)
Source: Embryotoxicity assay : Guernsey sea farm Ltd. Hatchery
Metamorphosis assay: SATMAR hatchery

Food: Embryotoxicity and metamorphosis assays: No food

Environmental conditions

Temperature:	Embryotoxicity assay: 22±1°C Metamorphosis assay: 22°C
Photoperiod:	Embryotoxicity and metamorphosis assays: No light
Aeration:	Embryotoxicity and metamorphosis assays: No aeration Embryotoxicity assay: 36h
Exposure duration	Metamorphosis assay: 24h

B. STUDY DESIGN AND METHODS**1. Preparation of the herbicide solutions**

The herbicide solutions were prepared with natural open sea water sterilised on a 0.22 µm membrane (Steritop®Millipore). The concentration ranges were prepared from stock solutions at 500 mg/L. For both endpoints, nominal concentrations corresponding to 0.1, 100 and 10000 µg/L of the chemicals (i.e., mecoprop and mecoprop-P) were verified (in duplicate) by ultraperformance liquid chromatography (UPLC) and MS-MS detection (in accordance with NF ENISO 11 369) using a UPLC Acquity with TQD detector (Waters– Milford, MA, USA) and a column Waters Acquity BEH C18 –2.1 mm × 150 mm, 1.7 µm. In accordance with the expected concentration, the samples were diluted or concentrated by solid phase extraction (Oasis – HLB 200 mg – Waters) before analysis. Moreover, the analyses were performed at the beginning and the end of the exposures to verify the variation in the tested concentrations during the period of the experiments. These analyses were performed once without embryos or larvae to avoid interaction between the physico-chemical and biological processes.

2. Embryotoxicity assay

Larvae were obtained using the standardised AFNOR procedure (AFNOR XP-T-906382) published in 2009. Briefly, male and female gametes were obtained by thermal stimulation (successive baths at 16 or 28°C). The egg density of the selected female was determined with a Mallassez counting cell. Twenty minutes after fertilisation, the embryos were distributed into plastic pillboxes containing 25 mL of natural sterilised sea water (0.22 µm, Steritop®Millipore) at a density of 60000 L⁻¹(corresponding to 1500 embryos per pillbox). After 36 h at 22 ± 1°C without feeding, aeration or light, embryos or D-shaped larvae were fixed using 0.5 mL of an 8% formalin solution. A minimum of 100 larvae was counted per replicate using an inverted binocular microscope at 400 × magnification (Leica®DMIRB). Observations enabled the calculation of rates of abnormality and the discrimination of types of abnormalities; four categories could be distinguished: normal larvae, mantle abnormality alone (hypertrophies), shell abnormality (with/without additional mantle abnormality), late arrested development and early arrested development (when cells could be distinguished and counted) (Mottier et al., 2013). The results of embryo-larval development in exposed organisms were expressed as net percentages of normal development (NPNe) adjusted for the controls (±SEM) (Mottier et al., 2013). For the three molecules tested, two experiments corresponding to two couples of genitors were conducted and, for each experiment, herbicide concentrations were tested in triplicate. Consequently, the minimum number of individuals examined for each concentration was 600. Herbicide concentrations ranged from 0.1 to 100000 µg/L with a factor of 10× between two consecutive concentrations, this range being tightened between 10000 and 100000 µg/L to precisely determine the EC₅₀ values (11 concentrations in total). CuSO₄·5H₂O (Alfa Aesar GmbH®; Karlsruhe, Germany) was used as a positive control with concentrations ranging from 20 to 100 µg/L (5 concentrations) according to the AFNOR procedure (AFNOR, 2009).

3. Metamorphosis assay

The aim of this endpoint was to assess the metamorphosis rate of pediveliger larvae (ready for metamorphosis) exposed to herbicides. These pediveliger larvae (21 days old) were kindly provided by the SATMAR (Société ATLantique de MARiculture) hatchery (Barfleur, France). Larvae were exposed in multi-well plates (12-wells, NUNC®; Penfield, New York, USA) in a final volume of 1.5 mL natural sterilised seawater (0.22 µm, Steritop®Millipore). Larval density was set between 50 and 80 larvae per well. To promote metamorphosis, epinephrine (Sigma Aldrich®) was added at a final concentration of 10^{-4} M (Coon and Bonar, 1987). Experiments were conducted for 24 h at 22°C without feeding, aeration or light. After 24 h, exposed larvae were observed using an inverted binocular microscope at 100 × magnification (Leica®DM IRB) to count dead larvae that exhibited tissue degradations and/or no movement. Following this first count, larvae were fixed using an 8% formalin solution. The metamorphosis rate was evaluated by counting metamorphosed versus non-metamorphosed larvae. A larva was considered metamorphosed when it presented an obvious loss of its velum, new shell growth and well-developed gills (Mottier et al., 2013). Dead larvae were very rarely observed, and metamorphosis rates were thus calculated by considering the percentages of non-metamorphosed versus metamorphosed ones. The results of the metamorphosis test in exposed organisms were expressed as net percentages of metamorphosis (NPM_e) (adjusted for the controls) (\pm SEM) (see Mottier *et al.*, 2013). Experiments were performed at least three times, and for each experiment, all herbicide concentrations were tested at least in triplicate. As for the embryotoxicity tests, the broad ranges of concentrations (between 0.1 and 100000 µg/L) were tightened, from 10000 µg/L for mecoprop and mecoprop-P (3 additional concentrations) and from 1000 to 10000 µg/L for 2-MCP (8 additional concentrations).

II. RESULTS AND DISCUSSION

FINDINGS

Preparation of the herbicide solutions: comparison between nominal and measured concentration

Table A-1 Pesticides concentrations (mean values in $\mu\text{g/L} \pm \text{SEM}$) measured for both endpoints at the beginning of the experiment and after 24 h or 36 h of exposure to mecoprop and mecoprop-P. (NC = Nominal Concentrations, MC = Measured Concentrations, % = percentage of differences between NC and MC)

Chemical	NC	Embryotoxicity				Metamorphosis rate			
		T0h		T36h		T0h		T24h	
		MC	%	MC	%	MC	%	MC	%
Mecoprop ($\mu\text{g/L}$)	0.1	0.115 \pm 0.005	15.5	0.099 \pm 0.006	1.25	0.098 \pm 0.009	2.25	0.105 \pm 0.012	5.25
	100	89.85 \pm 10.54	10.15	104.65 \pm 0.92	4.65	97.7 \pm 4.24	2.3	11.15 \pm 5.59	11.15
	10.000	10.490 \pm 975.81	4.9	9020 \pm 254.56	9.8	9940 \pm 509.12	0.6	9370 \pm 947.52	6.3
Mecoprop-P ($\mu\text{g/L}$)	0.1	0.10 \pm 0.01	2	0.101 \pm 0.01	1.25	0.089 \pm 0.01	11.5	0.084 \pm 0.01	15.75
	100	79.4 \pm 4.10	20.6	95.1 \pm 7.07	4.9	74.65 \pm 1.91	25.35	84.9 \pm 5.66	15.1
	10.000	8760 \pm 820.4	12.4	10.380 \pm 282.84	3.8	8310 \pm 240.42	16.9	7874 \pm 545.89	21.26

For both embryotoxicity and metamorphosis assay, the measured concentrations at T36h or T24h did not differ significantly from those recorded at T0. Consequently, it could be considered that organisms were exposed to constant concentrations during the whole experiments.

Moreover, the nominal concentration was compared with the measured concentration for the three compounds to control that no degradation of the herbicides occurred during the experiment. The differences were rather low. Nevertheless, the values of EC₅₀ in organisms exposed to mecoprop and mecoprop-P were re-calculated using measured concentration. The values of this ecotoxicological parameter were within the same order of magnitude than ones calculated with the nominal concentration and did not change the conclusions.

2-MCP could not be measured and data about the features (e.g. hydrolysis) of pesticides including 2-MCP generally refer to freshwater environments (surface freshwaters showing different pH values) and not to seawaters. Nevertheless, it can be hypothesised that the fate of 2-MCP in our experimental structures did not differ meaningfully from those of mecoprop and mecoprop-P. In fact, like both of its parent compounds, 2-MCP is highly water-soluble; data about its half-life are scarce, but hydrolysis (and photolysis) are estimated to be negligible (OECD, 2013).

Embryotoxicity and metamorphosis assays

Figure A-1 Net percentages of normal development (NPNe) (\pm SEM) in *C. gigas* embryo-larvae observed after 36 h of exposure to herbicides at concentrations ranging from 0.10 to 100000 μ g/L for mecoprop (A), mecoprop-P (B) and 2-methyl-4-chlorophenol (2-MCP) (C). The concentrations that do not share a letter are significantly different

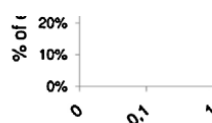


Figure A-2 Occurrence of the various types of abnormalities affecting embryo-larval development in *C. gigas* after 36 h of herbicide exposure in relation to the concentrations of three chemicals: mecoprop (A), mecoprop-P (B) and 2-methyl-4-chlorophenol (2-MCP) (C). NOR: normal D-shaped larvae; EMB: “old embryo”; SHEL + MASH: D-shaped larvae exhibiting shell and/or hinge abnormalities (with/without additional mantle abnormality); MANT: D-shaped larvae with a hypertrophied mantle in organisms exposed to MC

Whatever the tested contaminant, results related to the types of abnormalities indicated that the late arrested development was the predominant abnormality at high doses of chemicals, as individuals failed to reach the D-larva stage. The second most severe abnormality appeared to concern shell formation. Relatively high rates of shell deformities (including mantle abnormalities) were recorded for mecoprop (higher rate) and mecoprop-P (lower rate).

By contrast, in non-target organisms such as oyster larvae, exposure to mecoprop led to the calculation of a lower EC₅₀ value and thus was revealed to be more toxic than mecoprop ; this result suggests a greater impact of the (S)-(-) isomer than the (R)-(+) enantiomer on *C. gigas* embryo-larval stages. Other modes of action thus have to be considered, but the identification of such an alternative mode is difficult, notably because little is known about herbicide toxicity on non-target organisms or the precise mechanisms occurring during embryo-larval development in *C. gigas*.

3. Determination of endpoints

Table A-2 Ecotoxicological parameters calculated for the embryotoxicity tests (rates of abnormalities in D-shaped larvae) and for the metamorphosis tests (rates of pediveliger larvae metamorphosis) to 3 herbicide substances: mecoprop, mecoprop-P and 4-chloro-2-methylphenol. Ecotoxicological parameters are given for nominal and corrected concentration. ECX = effective concentration (in µg/L) which induces an effect on X% of the population (10% or 50%). na = not available

Endpoints	Parameters	Mecoprop	Mecoprop-P	2-MCP
Abnormality rates in D-shaped larvae (nominal concentrations)	EC ₁₀	32178.29	50489.47	8873.72
	EC ₅₀	42553.55	78853.55	10810.22
Abnormality rates in D-shaped larvae (measured concentrations)	EC ₁₀	26655.49	51361.05	na
	EC ₅₀	34479.17	80951.71	na
Metamorphosis rates of pediveliger larvae (nominal concentrations)	EC ₁₀	>100000	>100000	5603.40
	EC ₅₀	>100000	>100000	7199.79
Metamorphosis rates of pediveliger larvae (measured concentrations)	EC ₁₀	>100000	>100000	na
	EC ₅₀	>100000	>100000	na

2-MCP contaminant was revealed to be the most toxic with systematically EC₅₀ value superior for this degradation product in comparison with mecoprop and mecoprop-P.

For the embryotoxicity assay the EC₅₀ values were 79–81 and 34–43 mg/L for mecoprop and mecoprop-P respectively. For 2-MCP the calculated EC₅₀ was <11 mg/L.

Regarding the metamorphosis assay, the degradation product was also revealed to be more toxic than mecoprop and mecoprop-P. For the two parent molecules, the rate of metamorphosis remained high even at the highest concentration (100000 µg/L), and it was thus impossible to compute EC₅₀ values.

Finally, the three tested molecules could significantly affect embryo-larval development and metamorphosis but at concentrations far higher than those recorded in aquatic environments (maximum values of a few tens of µg/L^{1,2})

Other authors³ compared the toxicity of 12 pesticides, including mecoprop, to the survival and growth of *C. gigas* embryo-larval stages from fertilisation to nine days (veliger stage). They concluded that

¹ Fletcher et al., 1995

² Wittmer et al., 2010

there was a lethal effect of mecoprop on 50% of the studied larval population after nine days of exposure to a mecoprop concentration of 4200 µg/L. This relatively low value indicated a higher toxicity of mecoprop compared to the EC₅₀ values found in the current study for embryo-larval development and metamorphosis. His and Seaman's results are especially interesting because they demonstrated that longer exposures (9 days) could prove more injurious to oyster larvae than short exposures (24 or 36 h). With regards to shell growth for nine days, they reported a 10% height reduction in response to exposure to a rather low concentration, 130 µg/L and a hormesis effect at 50µg/L on *C. gigas* embryo-larval stage.

III. CONCLUSIONS

Mecoprop formulations and their degradation compound (2-MCP) were found to be “harmful” to oyster embryo-larval development according to the European Toxicity Classification system. The parent molecules could be considered “practically nontoxic” in terms of metamorphosis mechanisms, whereas 2-MCP was classified as “toxic”. Nevertheless, the EC₅₀ values allowing these classifications were much higher than the concentrations predicted or measured in coastal environments. Furthermore, the two enantiomers acted differently on non-target organisms (mecoprop more toxic to embryo-larval development) compared to target organisms (mecoprop-P exhibiting the herbicidal activity). The herbicides' toxicity appeared to depend not only on the molecule but also on the chirality of this molecule. For both endpoints, 2-MCP appeared to be more toxic than mecoprop and mecoprop-P, and the by-product was more toxic to pediveliger larvae (21 days old) compared to D-shaped larvae (36h old). In addition to active compounds and commercial formulations, it is thus important that regulations consider degradation products, which can be more harmful to the environment.

EC₅₀ determined in this study on oyster larvae could be listed with the endpoints already existing for mecoprop-P on aquatic organisms. Endpoints determined on oysters larvae in the current study do not change the conclusion of the ecotoxicological risk assessment of mecoprop since this substance is known to be slightly toxic to aquatic organisms.

Methodological quality

	Relevance	Reliability	Transparency and repeatability
Material	Study relevant to 3 substances: Mecoprop Mecoprop-P 2-MCP (biodegradation product)	Material well characterized and provided by Dr Ehrenstorfer GmbH	Material completely described and purity determined for each compound No CoA provided
Method	Embryotoxicity assay: Method AFNOR 2009 Broad range concentration tested for embryotoxicity and metamorphosis assays: 0.1 to 100000 µg/L	Comparison between nominal and measured concentration Comparison between concentration measured at T0h and T36h/T24h No method validation performed	Method used is accurately described in the original research report

³ His and Seaman, 1993

	Relevance	Reliability	Transparency and repeatability
Results and interpretation	Determination of EC ₅₀ for mecoprop, mecoprop-P and 2-MCP for metamorphosis assay and abnormalities rates in D-shaped larvae	Embryotoxicity: Minimum number of individuals examined for each concentration was 600. Metamorphosis: Experiments performed at least three times, with all herbicide concentration repeated at least in triplicate for each experiment	(seems correct)


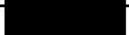

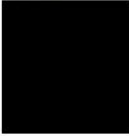






RMS comments:

The RMS has considered both the study report and the above applicant summary and reliability considerations. The paper summary and relevance/reliability assessment provided by the applicant have been detailed above. The RMS agrees with the applicant's assessment of reliability based on the current EU-guidance criteria. The critical EC₁₀ value for sub-lethal effects of mecoprop-P to the oyster *Crassostrea gigas* could be used to support the regulatory risk assessment for mecoprop-P, addressing data requirement 8.2.5.2. - Reproductive and development toxicity to an additional aquatic invertebrate species. **The critical study EC₁₀ for mecoprop-P is 50.49 mg a.s./L**, based on nominal concentrations. It is noted that this is almost identical to the 21-day NOEC for the same data requirement point from the available RMS-validated *D.magna* reproduction study (NOEC = 50 mg a.s./L), meaning this additional data would not adversely affect the outcome of the risk assessment for mecoprop-P.

References relied on, by data requirement according to (EU) 283/2013:

The references relied on list has been updated to include the newly submitted data relied on as well as those original submitted tests and studies (in *italics*) that are still considered relevant to support the application for renewal.

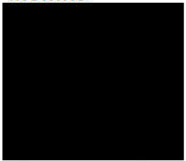






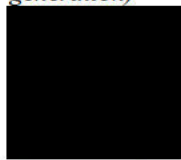

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.1.1.1		1986a	Avian single dose oral LD50 of MCP (mecoprop) TPH batch to the Bobwhite Quail (<i>Colinus virginianus</i>)	Y	N	N/A	MCP-P Task Force	In DAR (1998)

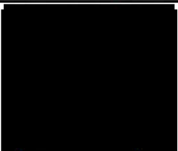





Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			 GLP Not published					
CA 8.1.1.1		1987	Avian single dose oral LD50 of MCPP D-Form to the Bobwhite Quail (Colinus virginianus) & amendment  Germany GLP Not published	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.1.1.1		1992a	R(+) 2-(2- methyl-4- chlorophenoxy) propionic acid dimethylamine salt (MCPP-P DMAS): 14 Day Acute Oral LD50 study in Bobwhite Quail  GLP Not published	Y	Y (but expired)	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.1.1.1		1995	Mecoprop-P acute oral toxicity (LD50) to bobwhite quail    	Y	N	New data	Nufarm	Submitted for purpose of renewal





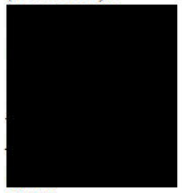

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			GLP Not published					
CA 8.1.1.2		1996	MCCP-P-DMA Salt – Avian Dietary LC50 test in chicks of the mallard duck (<i>Anas platyrhynchos</i> L.) GLP Not published	Y	N	New data submitted	MCCP- P Task Force	Submitted for purpose of renewal
CA 8.1.1.3		1999	<i>Technical Mecoprop-p DMA Effects on Reproduction in Japanese Quail after Dietary Administration</i> GLP Not published	Y	N	N/A	MCCP- P Task Force	<i>In Addendum II to DAR (July 2002)</i>
CA 8.1.2		1992b	<i>R(+)-2-(2- Methyl-4- chlorophenoxy) propionic acid dimethylamine salt (MCCP-P DMAS): 8-Day Acute Dietary LC50 Study in Bobwhite Quail</i>	Y	N	N/A	MCCP- P Task Force	<i>In DAR (1998)</i>

Data Point	Author(s)	Year	Title Company 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
			<div> <div></div> <div></div> GLP Not published </div>					
CA 8.1.2.1		1983a	<div> Report on the study of the acute oral toxicity in rats of CMPP (Mecoprop) (D-Form) <div></div> Report no. 84/028 Not published </div>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.1.2.1		1990a	<div> Mecoprop-P: acute oral toxicity study in the rat <div></div> GLP Not published </div>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.1.2.1		2009	<div> Acute dietary toxicity of MCPP-p in mice 25405 <div></div> <div></div> <div></div> GLP Not published </div>	Y	Y	New data submission	MCPP- P Task Force	Submitted for purpose of renewal
CA 8.1.2.1		1994a	<div> Mecoprop-P: Acute Oral LD50 in the rat. <div></div> </div>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			<i>GLP Not published</i>					
CA 8.1.2.1		1995	Mecoprop-P: acute oral neurotoxicity study in Wistar rats GLP Not published	Y	Y	Study not previously submitted in EU, conducted for US registration applications	MCPP- P Task Force	Submitted for purpose of renewal
CA 8.1.2.2		1986	<i>Report on the comparative study of the toxicity of the racemate and D-form of Mecoprop in rats after 7- week administration in the diet</i> <i>Not GLP Not published</i>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.1.2.2		1979	<i>Mecoprop, 3- month oral toxicity study in the rat (racemate, D- isomer)</i> <i>Not GLP Not published</i>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.1.2.2		1993	<i>Report on the study of the Oral Toxicity in BG6C3F1 Mice Administration</i>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			<i>in the diet for 3 months</i>  GLP Not published					
CA 8.1.2.2		2003	Mecoprop-P: oral (dietary administration) preliminary reproduction toxicity study in the rat     GLP Not published	Y	N	New study	Nufarm	Submitted for purpose of renewal
CA 8.1.2.2		1992	<i>Reproduction study with MCP-P in rats. Continuous dietary administration over 2 generations (2 litters in the first and 1 litter in the second generation)</i>  Not GLP Not published	Y	N	N/A	MCP-P Task Force	In DAR (1998)
CA 8.1.2.2		1993a	<i>Study of the prenatal toxicity of Mecoprop-P in rats after oral administration (gavage)</i>	Y	N	N/A	MCP-P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			 <i>Not GLP Not published</i>					
CA 8.1.2.2		1993b	<i>Study of the prenatal toxicity of Mecoprop-P in rabbits after oral administration (gavage)</i>  <i>GLP Not published</i>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.2.1		1984b	<i>Report on the study of the acute toxicity name of the test substance: CMPP (mecoprop)-D- form (Re. No. 154241) Animal species: rainbow trout (Salmo gairdneri Rich.)</i>  <i>Unpublished GLP 84/10031</i>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.2.1		1989	<i>Report on the study of the acute toxicity of mecoprop-P</i>	Y	N	N/A	MCPP- P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			Bluegill (<i>Lepomis macrochirus</i> RAF)  GLP Not published					
CA 8.2.1		1992a	Report on the study of the acute toxicity of mecoprop-P DMA salt on rainbow trout (<i>Oncorhynchus mykiss</i>). 12F0210/9150 56 92/11938  GLP Not published	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.2.1		1992b	Study report. Acute toxicity study on the Bluegill (<i>Lepomis macrochirus</i> RAF) of mecoprop-P DMA salt in a static system (96hours).  GLP Not published	Y	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.2.2		1993	Sublethal toxic effects on the rainbow trout	Y	N	N/A	MCPP- P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			<i>(Oncorhynchus mykiss Walbaum 1792) of mecoprop-P-acid in a flow through system (28 days); OECD 204. 42F0002/9151 34 (93/11174)</i> [REDACTED] GLP Not published					
CA 8.2.2	[REDACTED]	2015	Mecoprop-P acid: toxic effects to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in an Early-Life Stage toxicity test D92378 [REDACTED] GLP Not Published	Y	Y	New data submitted	Nufarm	Submitted for purpose of renewal
CA 8.2.2.3	[REDACTED]	1986	<i>Accumulation and elimination of 14C-mecoprop by Bluegill sunfish in a dynamic flow-through system.</i> [REDACTED] GLP Not published	Y	N	N/A	MCPP-P Task Force	In DAR (1998)
CA 8.2.4.1	Bell	1994	Mecoprop-P. Acute toxicity to <i>Daphnia magna</i> . Report No.:	N	Y (but expired)	N/A	MCPP-P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			RNP 447/941082 Huntingdon Research Centre Ltd GLP Not published					
CA 8.2.4.1	Elendt- Schneider, H	1991	Determination of the acute toxicity of Mecoprop-P (Reg.-Nr. 154 241) to the water flea <i>Daphnia magna</i> STRAUS 1/89/0280/ 50/1 BASF, Germany GLP Not published	N	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.2.5.1	Dohmen, G P	1993a	Effect of Mecoprop-P on the Reproduction of <i>Daphnia magna</i> STRAUS in a Chronic Toxicity Test P92-E117 (93/10844) BASF Aktiengesellsch aft, Germany GLP Not published	N	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 8.2.5.1	Müllerschö n, H ⁽¹⁾	1990	Influence of MCPP (as DMA salt) on the reproduction of <i>Daphnia magna</i> . CCR Project No. 167703 GLP: Y	N	N	N/A	MCPP- P Task Force	In DAR (1998)

Data Point	Author(s)	Year	Title Company 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
			<i>Published:N</i>					
CA 8.2.6.1	Dohmen, G P	1993b	<i>Effect of Mecoprop-P on the Growth of the Green Alga Pseudokirchneria subcapitata P91-E119 (93/10385) BASF Aktiengesellschaft, Germany GLP Not published</i>	N	N	N/A	MCPP- P Task Force	In DAR (1998)
CA 10.2.6.2	Armstrong, K	2000	<i>Mecoprop-P dimethylamine salt, Alga, growth inhibition test (72 h, EC50) (Anabaena flos-aquae) 17864/ 395831 (2000/100 0259) Inveresk Research, UK GLP Not published</i>	N	N	N/A	MCPP- P Task Force	In Addendum II to DAR (July 2002)
CA 8.2.6.2	Jenkins, C.A.	2007	<i>Mecoprop-P (DMA salt) algal growth inhibition assay Navicula ZZF0001/0631 20 Huntingdon Life Sciences Ltd GLP Not published</i>	N	N	New data	Nufarm	Submitted for purpose of renewal
CA 8.2.6.2	Burke, J.	2007	<i>Mecoprop-P (DMA salt) algal growth inhibition assay Skeletonema</i>	N	N	New data	Nufarm	Submitted for purpose of renewal

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			ZZF0002/0635 25 Huntingdon Life Sciences Ltd GLP Not published					
CA 8.2.7	Hoberg, J, R., and Witting, R.	1992	MCPP-P DMAS – Toxicity to The Duckweed <i>Lemna gibba</i> 92-3-4174 (92/5217) Springborn Laboratories Inc, USA GLP Not published	N	N	N/A	MCPP- P Task Force	In Addendum III to DAR (December 2002)
CA 8.2.8	Simmons, K	2015	Position Paper: Assessment of the presence or absence of the parent toxophore in o- cresol (metabolite of Mecoprop-P) Wyke_2015_0 43 Nufarm UK Ltd Not GLP Not published	N	Y	New data submitted	Nufarm	Submitted for purpose of renewal
CA 8.3.1.1	Weyman, G S	1999	MCPP-P DMA Acute contact and oral toxicity to honeybees 1149/24- D2145 Covance Laboratories, UK GLP Not published	N	N	N/A	MCPP- P Task Force	Included in Addendum II to DAR (July 2002)
CA 8.3.1.3	Kleebaum, K.	2014	Acute toxicity of Mecoprop-P technical acid	N	Y	New data submitted	Nufarm	Submitted for purpose of renewal

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			to honeybee larvae <i>Apis mellifera</i> L. under laboratory conditions (in vitro) 14 10 48 023 B BioChem agrar GLP Not published					
CA 8.3.1.4	Mack, P.	2012	LAF-74: a semi-field study to investigate residues in honeybee products and honeybee larvae (<i>Apis mellifera carnica</i> L.; Hymenoptera, Apidae) in Phacelia tanacetifolia in Germany in 2011 S11-02084 Eurofins Agroscience Services EcoChem GmbH GLP Not published	N	N	New data submitted	Nufarm	Submitted for purpose of renewal
CA 8.3.1.4	Franke, M	2013	Effects of CMPP-P K 600 g/L OAI on the honeybee <i>Apis mellifera</i> L. in a bee brood study under field conditions 12 10 48 001 B BioChem agrar GLP Not published	N	Y	New data submitted	Nufarm	Submitted for purpose of renewal
CP	Stevens, J	2014	Mecoprop-P K	N	Y	New	Nufarm	Submitted

Data Point	Author(s)	Year	Title Company 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
10.3.2.1/ 01			600 g/L – A rate-response laboratory bioassay of the effects of fresh residues on the parasitic wasp <i>Aphidius rhopalosiphii</i> (De Stefani- Perez) (Hymenoptera, Braconidae) NUF-14-1 Mambo-Tox Ltd, UK GLP Not published			guideline requirements		for purpose of renewal
CP 10.3.2.1/ 02	Fallowfield , L	2014	Mecoprop-P K 600 g/L – A rate-response laboratory bioassay of the effects of fresh residues on the predatory mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) NUF-14-2 Mambo-Tox Ltd, UK GLP Not published	N	Y	New guideline requirements	Nufarm	Submitted for purpose of renewal
CA 8.4.1	Stojano- witsch, M	2014	Mecoprop-P TGAI: sublethal toxicity to the earthworm <i>Eisenia fetida</i> Michaelsen (Haplotaxida, Lumbricidae) in artificial soil S13-00246 Eurofins Agroscience Services EcoChem	N	Y	New guideline requirements	Nufarm	Submitted for purpose of renewal

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			GmbH GLP Not published					
CA 8.6.2	Eley, R	2009a	Nufarm R(+)-MCCP herbicide: evaluation of the phytotoxicity and effect on seedling emergence and growth of terrestrial non target plants ACE-09-031 Agrochemex GLP Not published	N	N (Used in UK national assessment – data protection expires 27/01/19)	New data submitted	Nufarm	Submitted for purpose of renewal
CA 8.6.2	Eley, R	2009b	Nufarm R(+)-MCPP herbicide: evaluation of the phytotoxicity and effect on vegetative vigour of terrestrial non target plants ACE-09-032 Agrochemex GLP Not published	N	N (Used in UK national assessment – data protection expires 27/01/19)	New data submitted	Nufarm	Submitted for purpose of renewal
CA 8.6.2	Frank, P	2001a	BAS 037 32 H (MCPP-P DMA 600 g/l): A toxicity test to determine the effects of the test item on the seedling emergence of terrestrial plants 70 843 (2001/1007645) BASF	N	N (Used in UK national assessment – data protection expires 27/01/19)	N/A	MCPP- P Task Force	In Addendum II to DAR (July 2002)

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			<i>Aktiengesellschaft, Germany GLP Not published</i>					
CA 8.6.2	Frank, P	2001b	BAS 037 32 H (MCCP-P DMA 600 g/l): A toxicity test to determine the effects of the test item on the vegetative vigour of terrestrial plants 70 845 (2001/ 1007646) BASF Aktiengesellschaft, Germany GLP Not published	N	N (Used in UK national assessment – data protection expires 27/01/19)	N/A	MCCP-P Task Force	In Addendum II to DAR (July 2002)
CA 8.7	Hamm, R, T.	1987	Influence of Mecoprop on the growth of <i>Pseudomonas putida</i> BASF 87/10720 LR 2464 BASF, DE GLP: Y Not published	N	N	N/A	MCCP-P Task Force	In DAR (1998)
CA 8.8	Falk, S	2013	Toxicity to microorganisms activated sludge respiration inhibition test S13-00244 Eurofins Agroscience Services EcoChem GmbH GLP Not published	N	Y	New data submitted	Nufarm	Submitted for purpose of renewal
CA Section 9	Mottier et al.	2014	Effects of acute exposures to	N	N	N/A	Public	Submitted for purpose

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(lit. data)			Mecoprop, Mecoprop-p and their biodegradation product (2- MCP) on the larval stages of the Pacific oyster, Crassostera gigas. Aquatic Toxicology, 146, 165-175 Non GLP Published					of renewal

⁽¹⁾ Study not provided by notifier for renewal purposes so validity not confirmed by RMS. However lower endpoint reported in original DAR (1998) compared to submitted study (Dohmen, 1993a) and so study additionally utilised for risk assessment purposes.