Document Title

Summary of the ecotoxicological studies for Ethephon

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 8: Ecotoxicological studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

Date

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Author(s)

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2016-01-15	Initial document submitted for Annex I renewal Ethephon	M-544634-01-1
2017-07-21	Aquatic endpoints have been recalculated (p.22) and summaries of the recalculation have been included for the corresponding studies. Summary of publication included: [redacted]; [redacted]; [redacted]; 2011; M-520027-01-1; CA 8.2.4.1, p.27 Additional endpoints reported for study [redacted]; 2014; M-486043-01-1; CA 8.4.1, p62. Statement of endpoint recalculation for study [redacted]; 2015; M-538939-01-1; CA 8.4.2.1, p.74 included Amendments of studies on non-target plants (CA 8.6.2) with correct application rates Change of legal entity from Bayer CropScience AG to Bayer AG - Crop Science Division	M-544634-02-1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

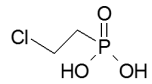
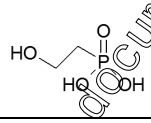
INTRODUCTION

Ecotoxicological data on ethephon was previously submitted in the EU Dossier (Baseline Dossier), which resulted in the existing Annex I inclusion under Directive 91/414/EEC. In the present Supplemental Dossier for renewal of approval of ethephon, individual study summaries are only included for studies which were not in the Baseline Dossier. To differentiate between additional studies and those evaluated in the previous review, the text in tables for the latter is stated in grey typeface.

A comprehensive search and review of the published literature for ethephon has been conducted. This is documented in the Supplemental Dossier. No publications were of sufficient quality and/or relevance for inclusion in Section CA 8.

The structural formulae of ethephon and a major metabolite (HEPA) are provided in Table 8-1.

Table 8 - 1: List of names, structures, and codes

Name and formula	**Codes used** – *IUPAC index name* / Other names / codes
Ethephon 	2-chloroethylphosphonic acid
HEPA 	2-hydroxyethylphosphonic acid/2-HEPA

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Studies on birds that have been conducted for the active substance are presented in Table 8.1.1- 1. Studies evaluated in the previous EU review are stated in grey text to distinguish them from new studies.

Table 8.1.1- 1: Ethephon: Endpoints from toxicity studies on birds

Test substance	Test species	Endpoints	Reference
Ethephon	Acute oral toxicity *Colinus virginianus*	LD_{50} 764 mg a.s./kg bw	LoEP[1] KCA 8.1.1.1/01 M-187798-01-1
	Acute oral toxicity *Anas platyrhynchos*	LD_{50} 1425 mg a.s./kg bw	LoEP KCA 8.1.1.1/02 M-187802-01-1
	Acute oral toxicity *Serinus canaria*	LD_{50} 1636 mg/kg bw	[redacted] (2013) KCA 8.1.1.1/03 M-457148-01-1
	Reproduction study *Coturnix japonica*	$NOEL_{repro}$ 100 mg a.s./kg diet 7.9 mg a.s./kg bw/d	LoEP KCA 8.1.1.2/01 M-203557-01-1
	Reproduction study *Anas platyrhynchos*	$NOEL_{repro}$ 1000 mg a.s./kg diet* 88 mg/kg bw/d	[redacted] (2014) KCA 8.1.1.3/02 M-474649-01-1
	Reproduction study *Colinus virginianus*	$NOAEL_{repro}$ 1000 mg a.s./kg diet* 87 mg/kg bw/d	[redacted] (2014) KCA 8.1.1.3/03 M-478412-01-1

* Highest treatment level.

CA 8.1.1.1 Acute oral toxicity to birds

For information on studies already evaluated during the previous EU review of ethephon, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoints from previously-evaluated studies are listed in Table 8.1.1- 1 in grey text.
A summary of a new study on acute oral toxicity to canary is presented below. The study was conducted to fulfil a requirement of the USEPA. The endpoint is stated in Table 8.1.1- 1 in black text.

Report: KCA 8.1.1.1/03; [redacted]; [redacted]; 2013; M-457148-01-1
Title: Toxicity of ethephon technical during an acute oral lD50 with canary (Serinus canaria)
Report No.: 07SRLS13C3
Document No.: M-457148-01-1
Guideline(s): EPA Ecological Effects Guidelines OCSPP 850.2100, Avian Acute Oral Toxicity Test (January 2012)
Guideline deviation(s): not specified
GLP/GEP: **yes**

Objective:

An acute oral toxicity test was conducted to derive the LD_{50} of ethephon to canary (*Serinus canaria*).

Methods:

The test item was ethephon 'Base 250' (analysed: 73.80% w/w a.s.; batch no. 03022F913-SA). Adult canaries were orally dosed with ethephon based on body weight at dose levels of 0, 125, 250, 500,

[1] EFSA Scientific Report (2008) 174: Conclusion on the peer review of ethephon; List of Endpoints

1000, and 2000 mg a.s./kg bw. Ten birds per dose level (five males and five females) were randomized by body weight into each treatment level on experimental Day -1. Birds were capsule-dosed on Day 0 and subsequently monitored for 14 days. All feed and water were provided *ad libitum*. Adult body weights were measured on experimental Day -1, Day 7, and Day 14. Feed consumption and clinical observations occurred daily.

Results:

Mortality & Clinical Observations

Mortality was observed of one bird at 1000 mg a.s./kg bw and seven birds at 2000 mg a.s./kg bw. All mortality occurred two hours following dosing. Lethargy and diminished reaction to stimuli (hypo-reactivity) were observed in all treatment groups. Ataxia (loss of muscular coordination), hypo-reactivity to stimuli, and immobility were observed at 500, 1000, and 2000 mg a.s./kg bw. Severity and prevalence of clinical observations were primarily dose dependent. One bird at 125 mg a.s./kg bw had minimal observed effects (lethargy and hypo-reactivity), whilst the other nine showed no effects. All surviving birds recovered by Day 1 from observed symptoms.

Body Weight & Feed Consumption

Body weight measurements (Day 0, Day 7 and Day 14), changes in body weight (Day 0 to Day 7, Day 7 to 14, and Day 0 to Day 14), and individual food consumption measurements (Day 0 to Day 7, Day 7 to Day 14, Day 0 to Day 14) were not significantly different when treatment groups were compared to the control group. Comparisons were made among all surviving birds by treatment group.

Conclusion:

The acute oral LD_{50} for canary exposed to ethephon was 1636 mg a.s./kg bw (95% CL = 1226 to 2476 mg a.s./kg bw).

CA 8.1.1.2 Short-term dietary toxicity to birds

No additional studies were performed. For information on studies already evaluated during the first EU review of ethephon, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and in the Monograph. This type of study is no longer used in the Tier 1 risk assessment. Hence, the endpoints are not listed in Table 8.1.1- 1.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

For information on studies already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. Summaries of two new studies on the reproductive toxicity to birds are presented below. The studies were conducted to fulfil a requirement of the USEPA. Endpoints are listed in Table 8.1.1- 1.

Report:	KCA 8.1.1.3/02; [redacted]; [redacted] M.; 2014; M-474649-01-1
Title:	Toxicity of ethephon (Base 250) on the reproduction of the mallard duck (Anas platyrhynchos)
Report No.:	07SRLS13C4
Document No.:	M-474649-01-1
Guideline(s):	OECD Guideline No. 206. Avian Reproduction Toxicity Test; EPA Ecological Effects Guidelines OPPTS 850.2300 Avian Reproduction Test
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

The purpose of this study was to evaluate the effects of dietary exposure to ethephon on the health and reproductive capacity of mallard ducks (*Anas platyrhynchos)*.

Material and Methods:

The test substance was ethephon 'Base 250' (73.80% w/w ethephon; Batch no. 03022F913-SA). The study exposed adult mallard ducks for approximately 20 weeks to nominal dietary concentrations of 0 (control), 111, 333 and 1000 mg a.s./kg feed. Mallard ducks were 20 weeks old at experimental start with 15 pairs of birds at each treatment level. Birds were observed for mortality, abnormal behaviour and signs of toxicity. Adult body weight and feed consumption were measured. Gross pathology was conducted. Reproductive parameters, as well as hatchling health, growth and survival, were examined. The biological portion of the study was conducted from 11 September 2012 to 12 March 2013.

Results:

Dietary Concentrations

The nominal concentrations were 0 (control), 111, 333, and 1000 mg a.s./kg feed. The average measured concentrations of ethephon for Weeks 1, 5, 10, 15, and 20 were 0, 106, 300, and 955 mg a.s./kg feed representing percent nominal values of 95%, 90%, and 95%, respectively. These values correspond to daily dietary dose levels of 0, 10, 27, and 88 mg a.s./kg bw/day, respectively. A summary of the dietary concentrations is included in the following table.

Feed Analysis Summary of Ethephon				
Nominal Dietary Level (mg a.s./kg feed)	Measured Dietary Level (mg a.s./kg feed)	Percent of Nominal	Daily Dietary Dose (mg a.s./kg bw/day)*	Food Consumption (% mean bw)
0 (control)	0	-	-	-
111	106	95 %	10	9.4
333	300	90 %	27	9.1
1000	955	95 %	88	9.3

* Daily Dietary Dose based on measured concentrations.

Adult Bird Mortality & Clinical Observations

Mortality occurred for one adult bird in the 1000 mg a.s./kg feed level which was not considered treatment related. There were no significant clinical symptoms or compound related effects observed during the study. Several adult birds were observed in the control and treatment levels with feather loss and minor abrasions as a result of normal cage wear for laboratory birds.

Adult Bird Bodyweight

The adult body weights were measured prior to dosing and every other week up to the egg production phase (i.e. Weeks 3, 5, 7, 9) and prior to adult sacrifice. No effects were observed for adult male or female termination bodyweights or bodyweight gain. The NOEL for the adult bodyweight or bodyweight gain was 1000 mg a.s./kg feed.

Adult Bird Feed Consumption

Adult bird food consumption was measured weekly over a 20-week period. There were no statistically significant differences at any treatment level compared to the control for adult bird food consumption and the NOEL was 1000 mg a.s./kg feed.

Adult Bird Necropsy

Necropsy observations of adult birds revealed feather loss in all treatment levels and the control. These observations were due to normal cage wear for laboratory reared mallard ducks in the reproductive phase. A small number of female birds were found with regressed ovaries as follows: control (1), 333 mg a.s./kg feed (1), and 1000 mg a.s./kg feed (1). All male reproductive organs appeared normal for all treatment levels.

The results for reproductive parameters are given in Table 8.1.1-2 as magnitude and in Table 8.1.1-3 as percentages. Results are based on 14 hens (i.e. pairs) for the 1000 mg a.s./kg feed treatment group due to a single female mortality. Results for egg viability (and consequent endpoints) are based on 14 hens (i.e. pairs) for the 111 and 333 mg a.s./kg feed treatment groups due to no eggs being viable for a single hen (i.e. pair) in each group.

Table 8.1.1- 2: Results of a reproduction study on Mallard duck for ethephon

Mallard Reproduction Study Reproductive Endpoint Totals (per hen) [a]					
Reproductive Parameter	Nominal Dietary Concentration (mg a.s./kg feed)				
		Control	111	333	1000
Number of Eggs Laid	Mean	60.6	57.2	52.7	57.7
	SD	10.2	9.55	12.7	10.2
	N	15	15	15	14
Number of Eggs Cracked	Mean	0.67	0.60	0.80	0.36
	SD	0.82	0.91	2.31	0.50
	N	15	15	15	14
Number of Eggs Set	Mean	54.5	51.3	46.9	52.1
	SD	10.5	9.8	13.4	10.2
	N	15	15	15	14
Number of Viable Embryos	Mean	51.5	47.8	40.2	49.2
	SD	10.3	9.9	17.3	10.4
	N	15	14	14	14
Number of Live Embryos	Mean	51.2	47.6	39.6	49.1
	SD	10.5	9.9	17.6	10.2
	N	15	14	14	14
Number Hatched	Mean	45.6	40.9	32.2	43.2
	SD	9.6	12.4	15.0	12.1
	N	15	14	14	14
Number of 14-Day Survivors	Mean	45.3	40.4	31.6	42.9
	SD	9.8	12.2	14.8	11.8
	N	15	14	14	14

[a] Values from SAS statistical output

Table 8.1.1- 3: Results of a reproduction study on Mallard duck for ethephon, expressed as percentages

Mallard Reproduction Study Reproductive Endpoint Percentages (per hen) [a]					
Reproductive Parameter	Nominal Dietary Concentration (mg a.s./kg feed)				
		Control	111	333	1000
Eggs Not Cracked of Laid	Percent	98.9	98.9	98.2	99.3
	SD	1.2	1.7	5.5	1.0
Eggs Set of Eggs Laid	Percent	89.6	89.4	88.0	90.1
	SD	5.1	4.1	8.3	2.3
Viable Embryos Of Eggs Set	Percent	94.5	93.4	85.8	94.4
	SD	5.1	5.5	24.7	8.6
Live Embryos Of Viable Embryos	Percent	99.4	99.6	97.5	99.8
	SD	1.5	1.2	5.4	0.6
Number Hatched Of Eggs Laid	Percent	75.2	70.7	60.3	73.9
	SD	8.6	13.2	22.3	13.9
Number Hatched Of Eggs Set	Percent	83.9	79.2	68.0	82.0
	SD	7.9	14.2	23.1	15.0
Number Hatched Of Live Embryos	Percent	89.5	85.1	83.0	86.9
	SD	7.9	13.8	15.7	13.1
14-Day Survivors Of Eggs Set	Percent	83.2	78.4	66.5	81.5
	SD	8.2	13.7	25.1	14.8
14-Day Survivors of Number Hatched	Percent	99.2	99.1	97.7	99.4
	SD	1.6	1.9	3.0	1.1

[a] Values from SAS statistical output.

Egg Reproductive Effects

There were no statistically significant adverse effects for the following egg reproductive endpoints: number of eggs laid, percent eggs set of eggs laid, number of eggs cracked, percent eggs not cracked of laid, eggs set, eggshell strength, and eggshell thickness. The NOEL for these endpoints was 1000 mg a.s./kg feed.

Embryo Reproductive Effects

The 333 mg a.s./kg feed level was statistically significantly different from the controls for the number of viable embryos and the number of live embryos. No significant differences occurred for the percent viable embryos of eggs set and the percent live embryos of viable embryos. As no statistically significant differences from the control were seen at 1000 mg a.s./kg feed and all parameters at 333 mg a.s./kg food were within the range of historical control values, the NOEL for these endpoints was determined to be 1000 mg a.s./kg feed.

Hatchling Effects

The 333 mg a.s./kg feed level was statistically significantly different from the control for the number hatched, percent hatched of eggs set, and number of 14-day hatchling survivors. No significant difference occurred for the following: percent hatched of eggs laid, percent hatched of live embryos, percent 14-day survivors of eggs set, and percent 14-day survivors of hatched. As no statistically significant differences from the control were seen at 1000 mg a.s./kg feed and all parameters at 333 mg a.s./kg food were within the range of historical control values, the NOEL for these endpoints was determined to be 1000 mg a.s./kg feed.

Hatchling Body Weight

There were no statistically significant differences at any treatment level as compared to the control for initial hatchling weights and 14-day survivor body weights. There were no hatchlings produced from the study that were observed to have any abnormal symptoms. Minor mortality (≤ 2%) was observed in the hatchling phase among all treatments and control. The NOEL for these endpoints was 1000 mg a.s./kg feed.

Conclusion:

The NOEL for both parental toxicity and reproduction endpoints of mallard ducks exposed to ethephon was 1000 mg a.s./kg feed (nominal) with a mean measured concentration of 995 mg a.s./kg feed. This was the highest treatment level in the study.

The calculated mean Daily Dietary Dose (DDD) in the 1000 mg/kg feed treatment group was 88 mg a.s./kg bw/day.

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Report:	KCA 8.1.1.3/03; [redacted]; 2014; M-478412-01-1
Title:	Ethephon: Reproductive toxicity test with the northern bobwhite (Colinus virginianus) - Ethephon technical (Base 250)
Report No.:	XY4711
Document No.:	M-478412-01-1
Guideline(s):	OECD Guideline No. 206. Avian Reproduction Toxicity Test; EPA Ecological Effects Guidelines OPPTS 850.2300 Avian Reproduction Test
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:

To evaluate the reproductive effects of dietary exposure of adult northern bobwhite (*Colinus virginianus*) to ethephon over a period of 24 weeks, and the effects on adult health, body weight, feed consumption and reproductive success, as evaluated by the number of eggs laid, eggshell thickness, egg fertility, embryo viability, hatch rates, hatchling survival and hatchling weight.

Material and Methods:

The test substance was ethephon technical concentrate (73.80% w/w ethephon; Batch no. 03022F913-SA). Test organisms were young adults held in cages measuring 81 cm wide x 91 cm deep x 20.5 to 25 cm high. For use in the study, 144 individuals (72 males and 72 females) were indiscriminately selected. There was 1 male and 1 female per cage, and 18 cages per treatment level and control. Following 14 days of acclimation, adult birds were exposed to feed treated with ethephon for 24 weeks. This included 10 weeks of exposure to treated feed prior to photo-stimulation, 4 weeks during photo-stimulation, and 10 weeks during which eggs were collected.

Adult room conditions ranged from 22 to 27 °C and 45 to 70 % relative humidity during acclimation, and 20 - 28 °C and 31 – 82 % relative humidity during the experimental period. Light intensity in the adult test room averaged 14.7 foot-candles, with 7 hours light and 17 hours darkness during the pre-photo-stimulation period, and 17 hours light and 7 hours darkness from photo-stimulation until adult termination. Brood rearing conditions ranged from 29 to 41 °C, with the range generally decreasing over time, and 16 – 60 % relative humidity. The light schedule in the brooding room was 14 hours light and 10 hours darkness.

The nominal feed concentrations tested were as follows: 0, 111, 333 and 1000 mg a.s./kg feed. During the experimental period, adult food consumption (per pen) was measured weekly, or more often as food was added. Food consumption by the hatchlings was not measured. The test was conducted with 18 replicates per treatment level.

Mortality and signs of intoxication were assessed daily. Body weight was measured for each adult eight times during the course of the study: at the start of acclimation (cage assignment), immediately prior to treatment initiation, at the end of weeks 2, 4, 6, 8 and 10 of the pre-laying period, and at post-

egg collection upon adult euthanasia. The food consumption was calculated from weighing the residual food weekly throughout the study. Egg incubation was initiated weekly (after start of reproduction). During the course of the study on one day out every 14 days all eggs were retained at each treatment level for measurements of shell thickness. Candling of all eggs was done on day 11 and day 18 of incubation to assess embryo development and survival, respectively. Body weight of hatchlings was measured after completion of hatching and after 14 days. Food was analysed in order to verify the homogeneity and the concentrations of the test item and its ambient stability in the feeder.

Results:

Mean dietary concentrations as measured in diet verification and homogeneity samples yielded mean concentrations across all analysed and reported diet mixture analyses, and across all dietary concentrations of 88.9 – 102.0 % of nominal. Since measured concentrations were within ± 20% of nominal, per guideline requirements, the diet preparation method is considered to have achieved satisfactory concentration of the test substance in the diet. The results of this study are therefore based on nominal concentrations. Calculated coefficient of variation values ranged from 2.6 – 7.2 % across all analysed and reported diet mixture analyses.

Biological Findings:

The results for reproductive parameters are given in Table 8.1.1- 4 expressed as magnitude and Table 8.1.1- 5 expressed proportionately.

Table 8.1.1- 4: Results of Bobwhite quail (*Colinus virginianus*) Reproduction Test with Ethephon

Parameter	Average per Hen, by Treatment Group (mg a.s./kg feed)			
	Control	111	333	1000
Number of laying pairs	18	17[a]	18	18
Average total eggs laid per hen[b]	48	48	54	47
Average total eggs cracked per hen	0.3	0.7	0.3	0.6[c]
Average total eggs incubated per hen	44	43	49	39
Average total viable eggs per hen	41	40	46	37
Average total surviving embryos per hen	41	40	46	37
Average total number of successful hatchlings per hen	39	37	43	34
Average total number of 14-day old offspring survivors per hen	37	35	42	33
Mean hatchling weight (g)	7.5	7.7	7.7	7.6
Mean 14-day old survivor weight	26.6	27.3	27.7	28.0

[a] Due to injury of the male the male and female in Cage 64 were euthanized prior to photostimulation, so is excluded from all egg production analyses. The pair in Cage 66 failed to produce any eggs during the study, but was included in the data set analyzed, with a value of 0 for all parameters.

[b] Total egg collection days possible = 70 days.

[c] Significantly higher than the control according to Jonckheere's test (ANOVA p = 0.324; Jonckheere p = 0.032).

Table 8.1.1- 5: Results of Bobwhite quail (*Colinus virginianus*) Reproduction Test with Ethephon expressed as proportions (%)

Parameter	Treatment Group (mg a.s./kg feed)			
	Control	111	333	1000
Number of eggs laid per hen per day	0.7	0.7	0.8	0.7
% of Non-cracked eggs of eggs laid	99.53[b]	98.41	99.46	98.66
% of Eggs incubated of eggs laid	86	88	85	83
% Viable eggs of eggs incubated	89	93	93	96
% Surviving embryos of viable eggs	99	99	100	99
% Successful hatches of eggs laid	72	74	75	71
% Successful hatches of eggs incubated	84	84	88	84
Successful hatches of surviving embryos	95	90	94	89
% 14-day old offspring survivors of eggs incubated	79	81	86	92
% 14-day old offspring survivors of successful hatches	94	96	98	98

[a] Significantly lower than the control according to Jonckheere's test (ANOVA p = 0.295; Jonckheere p = 0.028)

[b] *Evaluator comment:* In the summary table in the study report the value is stated as 100%, but this is an error because there were 5 cracked eggs in the control group, being 0.6% of the total number of eggs laid (as stated on p 35 of the study report).

For measurement of eggshell thickness, the number of eggs assessed were: Control = 65; 111 mg a.s.kg/feed = 54; 333 mg a.s./kg feed = 70; 1000 mg a.s./kg feed = 56. The results for eggshell thickness are summarised in Table 8.1.1- 6.

Table 8.1.1- 6: Summary of results for eggshell thickness

Parameter	Eggshell Thickness (mm) Summary by Treatment Group (mg a.i./kg feed)			
	Control	111	333	1000
N[a]	16	16	17	16
Mean	0.196	0.194	0.191	0.188[b]
Standard Deviation	0.012	0.011	0.009	0.013

[a] N represents number of hens in each group that produced eggs from which EST was measured.
[b] Significantly lower than the control according to Williams' test (ANOVA p = 0.231; Williams p = 0.035).

A statistically significantly lower average eggshell thickness (4.1% less than control) and a statistically significant higher number of cracked eggs (mean total of 0.6 eggs per hen in treatment and 0.3 in control) compared to the control group were observed in the 1000 mg a.s./kg feed treatment group. These differences were not considered by the study author to be biologically-relevant adverse effects because: 1) There were no statistically significant differences from the control in number of eggs hatched or number of 14-day old surviving chicks. 2) The number and percentage of cracked eggs at this highest treatment level fell within or was lower than historical control data. According to OECD test guideline 206 the "normal" value for cracked eggs is given as 0.6 – 2% for bobwhite quail studies. This indicates that in the present study the percentage of cracked eggs was at the low end of the

normal background range. 3) The eggshell thickness difference from the control (4.1%) and cracked egg percentage (0.6%) at 1000 mg a.s./kg bw were both <5%.

Based on a daily percent food consumption compared with bodyweight of 8.7% in the 1000 mg a.s./kg feed treatment group, the DDD in this group was calculated by the study author to be 87.0 mg a.s./kg bw/day.

Based on a daily percent food consumption compared with bodyweight of 8.7% in the 333 mg a.s./kg feed treatment group, the DDD in this group was calculated by the study author to be 29.0 mg a.s./kg bw/day.

Conclusion:

Based on a purely statistical analysis, the LOEC is 1000 mg a.s./kg feed (87.0 mg a.s./kg bw/day), and the NOEC is 333 mg a.s./kg feed (29.0 mg a.s./kg bw/day). However, the No Observable Adverse Effect Concentration (NOAEC) is considered to be 1000 mg a.s./kg feed and the Lowest Observed Adverse Effect Concentration (LOAEC) is considered to be > 1000 mg a.s./kg feed.

Evaluator comment: The eggshell thickness difference from the control (only 4.1%) and cracked egg percentage (only 0.6%) at 1000 mg a.s./kg bw were both judged as not biologically-relevant by the study author. Hence, the *NOAEL* of 87.0 mg a.s./kg bw/day is considered the relevant value for the risk assessment.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Endpoints from studies on mammals that have been conducted for the active substance are presented in Table 8.1.2- 1. All relevant studies were evaluated during the previous EU review. Hence, all endpoints are stated in grey text.

Table 8.1.2- 1: Ethephon: Endpoints from toxicity studies on mammals

Test substance	Test species	Endpoint		Reference
Ethephon (Base 25%)	Acute oral LD_{50} Rat	LD_{50}	1564 mg a.s./kg bw	LoEP KCA 5.2.1/01 M-187938-01-1
Ethephon	Reproduction Rat	NOAEL	22.8 mg a.s./kg bw/d	LoEP KCA 5.6.1/01 M-187771-01-1

CA 8.1.2.1 Acute oral toxicity to mammals

For information on studies already evaluated during the previous EU review, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoint is stated in Table 8.1.2- 1 in grey text.

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

For information on studies already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph and the Addenda generated during the EU review. The endpoint is stated in Table 8.1.2-1 in grey text.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

Substances with a high bioaccumulation potential could theoretically pose a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log P_{ow} >3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. As the log P_{ow} of ethephon is less than the trigger, no evaluation of secondary poisoning is needed.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

Information on effects of ethephon on reptiles or amphibians is not available. Risk to birds and mammals is assessed in MCP Section 10.1.

CA 8.1.5 Endocrine disrupting properties

Based on the analysis of the complete toxicological data set, there is no evidence of any endocrine disrupting potential of ethephon in mammals. Likewise in studies with birds, fish and other aquatic organisms no indication of an endocrine activity was found. Therefore it is concluded that Ethephon has no endocrine disrupting activity in environmental organisms. Further special testing for endocrine disrupting properties is therefore not warranted. Further details are provided in a Position Paper which is included in Appendix 1.

CA 8.2 Effects on aquatic organisms

In order to complete the aquatic risk assessment and to address new data requirements according to Regulation No. 1107/2009, additional studies have been performed compared with the data available for the previous EU review. These additional studies are summarized in the following section. For studies submitted during the previous EU review, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

The degradation pathways in soil, and water/sediment systems, are given in the two figures below. For further details please refer to Section 7: "Fate and behaviour in the environment".

Figure 8.2- 1: Degradation pathway of ethephon in soil

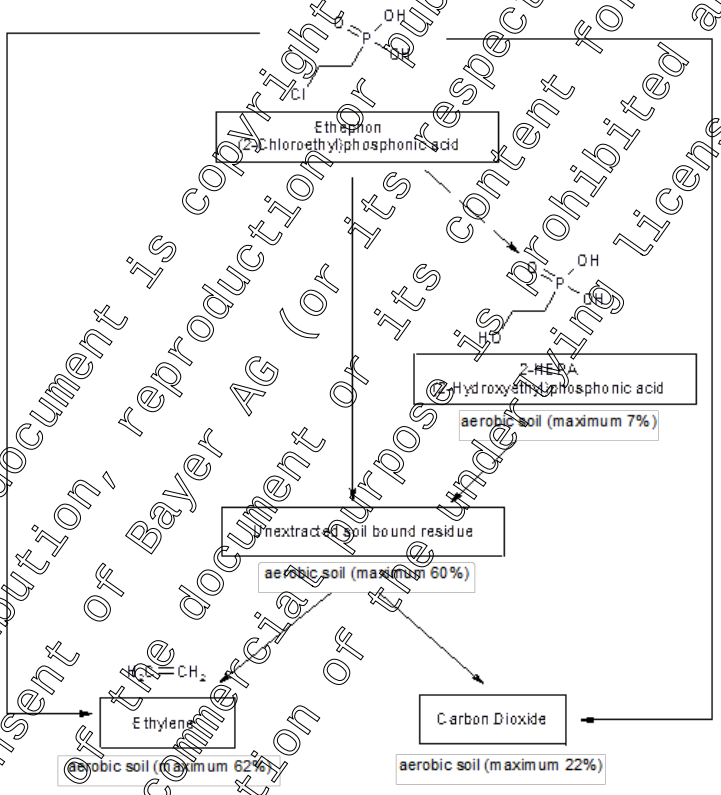
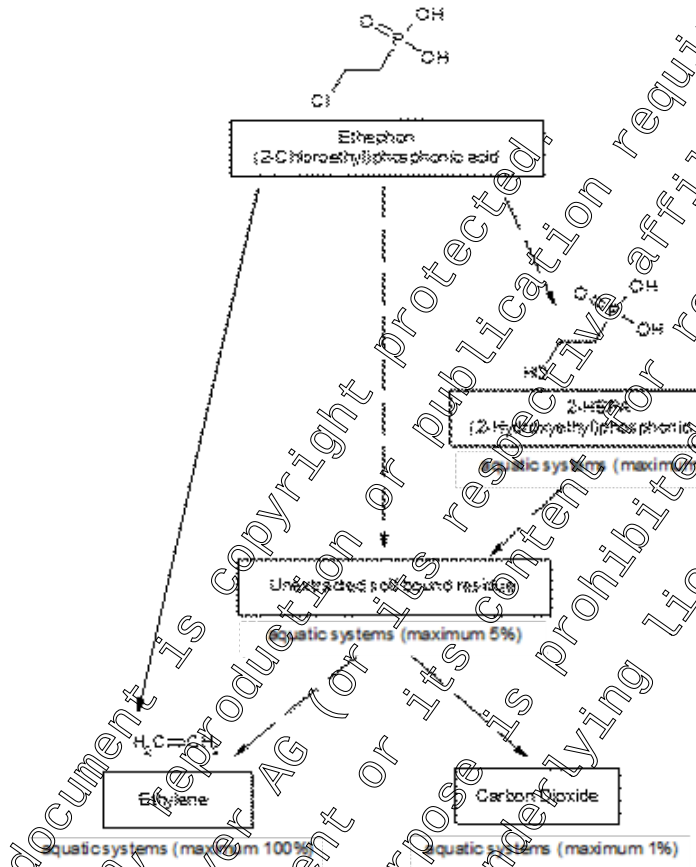


Figure 8.2- 2: Degradation pathway of ethephon in water/sediment systems



Studies on aquatic organisms have been conducted for the active substance. The endpoints from these studies are presented in Table 8.2-1. Endpoints from studies evaluated during the previous EU review are stated in grey text to distinguish them from the additional studies. The following bullet points provide the rationale for conducting each of the additional studies:

- Acute toxicity to *Cyprinodon variegatus* (sheepshead minnow) ([redacted], 2013): Study conducted to fulfil a requirement of US EPA, and is submitted now for completeness.
- Acute toxicity to *Daphnia magna* ([redacted], 2015): Conducted because a previous acute toxicity study on *D. magna* was judged as unreliable in the previous EU review.
- Acute toxicity (shell growth) to *Crassostrea virginica* (Eastern oyster) ([redacted], 1989): Standard study on marine species as required by US EPA. Submitted now for completeness.
- Algal growth inhibition of *Skeletonema costatum* ([redacted], 1990): Standard study on this marine species as required by US EPA. Submitted now for completeness.
- Algal growth inhibition of *Navicula pelliculosa* ([redacted], 2015): To provide a study on an additional algal species which is fully-compliant with OECD Guideline No. 201 (2006).
- Growth inhibition of aquatic macrophyte *Myriophyllum spicatum* ([redacted], 2015): Conducted to satisfy point 8.2.7 of the active substance data requirements under Regulation 1107/2009.

Ecotoxicological endpoints

Table 8.2- 1: Ethephon: Endpoints from toxicity studies on aquatic organisms

Test substance	Test species	Endpoint		Reference
Ethephon	Fish, acute, *Cyprinus carpio*	LC_{50}	>100 mg a.s./L	LoEP KCA 8.2.1/03 M-187823-01-1
	Fish, acute, *Cyprinodon variegatus*[1]	LC_{50}	>102 mg a.s./L	[redacted] (2013) KCA 8.2.1/04 M-444829-01-1
	Fish, chronic (ELS) *Pimephales promelas*	NOEC	45 mg a.s./L[3]	LoEP KCA 8.2.2.1/01 M-205148-01-2
	Invertebrate, acute *Daphnia magna*	EC_{50}	>90.4 mg a.s./L	[redacted] (2015) KCA 8.2.4.1/02 M-524938-01-1
	Invertebrate, acute *Crassostrea virginica*[b] (Eastern oyster)	EC_{50} shell growth	60 mg a.s./L	[redacted] (1989) KCA 8.2.4.2/01 M-187969-01-1
	Invertebrate, chronic *Daphnia magna*	21d LC_{50} NOEC EC_{10} EC_{20}	>160 mg a.s./L[2] 67 mg a.s./L 122.7 mg a.s./L[4] 151.1 mg a.s./L[4]	LoEP KCA 8.2.5.1/01 M-187833-01-1
	Algae, growth inhibition *Chlorella vulgaris*	E_bC_{50}	20.9 mg a.s./L	LoEP KCA 8.2.6.1/01 M-187835-01-1
	Algae, growth inhibition *Selenastrum capricornutum*	E_bC_{50}	>1.4 mg a.s./L[5]	LoEP KCA 8.2.6.1/02 M-187839-01-1
	Algae, growth inhibition *Navicula pelliculosa*	E_bC_{50}	>1.5 mg a.s./L[6]	LoEP KCA 8.2.6.1/03 M-187837-01-1
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_bC_{50}	7.1 mg a.s./L	LoEP KCA 8.2.6.1/04 M-236983-01-1
	Algae, growth inhibition *Navicula pelliculosa*	E_rC_{50}	>2.86 mg a.s./L	[redacted] (2015) KCA 8.2.6.1/05 M-534339-01-1
	Algae, growth inhibition *Skeletonema costatum*[1]	E_bC_{50}	>1.8 mg a.s./L[6]	[redacted] (1990) KCA 8.2.6.1/06 M-187843-01-1
	Algae, growth inhibition *Anabaena flos aquae*	E_bC_{50}	>1.8 mg a.s./L[5]	LoEP KCA 8.2.6.2/01 ~~M-236983-01-1~~ M-187841-01-1
	Aquatic plants, growth inhibition *Lemna gibba*	E_bC_{50}	>1.6 mg a.s./L[7]	LoEP KCA 8.2.7/01 M-187845-01-1
	Aquatic plants, growth inhibition *Myriophyllum spicatum*	E_rC_{50}	>100 mg a.s./L	[redacted] (2015) KCA 8.2.7/02 M-537257-01-1

[1] Estuarine/marine species, tested in salt water.
[2] LC_{50} for parental *Daphnia*. This is the agreed *acute* endpoint from the previous EU review (at that time the 48h acute study was deemed invalid). A new acute toxicity study has been conducted for the current EU review.

[3] As requested by the RMS, EC_{10} and EC_{20} values should be determined as additional endpoints to this study. However, due to the lack of a concentration response, it was not possible to derive valid EC_{10} and EC_{20} from the results of the study.

[4] As requested by the RMS, EC_{10} and EC_{20} values should be determined as additional endpoints to this study. According to the new aquatic Guidance Document (EFSA, 2013, Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290), the EC_{10} is the more relevant endpoint compared to the NOEC and is therefore used in the aquatic risk assessment.

[5] The study was considered valid at the time of the original inclusion of ethephon. However, according to the current test guidelines and due to statistical reasons, a re-evaluation of the study endpoints is not reasonable. Results of the study are not used in the risk assessment.

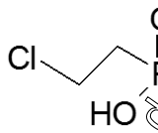
[6] The RMS asked to calculate additional endpoints for growth rate and yield. This limit study was considered valid at the time of the original inclusion of ethephon. However, endpoint recalculation is not possible due to a high coefficient of variation exceeding the validity criterion of 35%. In addition, no EC_{50} value can be derived from a limit test. Results of the study are not used in the risk assessment.

[7] The RMS requested to calculate the endpoints for growth rate and yield. However, due to mathematical reasons, it was not possible to derive valid endpoints from the results of the study.

Note on metabolite HEPA:

HEPA is classed as a 'major' metabolite of ethephon in soil, having been detected at 10.6% (i.e. >10%) of applied radioactivity in a soil photolysis study on ethephon (MCA Section 7). In accordance with the EFSA Aquatic Guidance Document (2013), the 'relevance' of HEPA to the risk assessment needs to be considered. The molecular structures of ethephon and HEPA are shown below.

Ethephon:



HEPA:

Given that the structure of HEPA is very similar to ethephon (which is of low toxicity, as shown in Table 8.2-1) and the molecule has no toxophore, HEPA is concluded to be 'non-relevant' for the risk assessment. Therefore, *a priori*, by reference to the EFSA Aquatic Guidance Document (2013), the acute and chronic toxicity of HEPA can be assumed to be equal to the toxicity of ethephon for all first tier taxonomic groups. As such, aquatic toxicity tests on HEPA are not required.

CA 8.2.1 Acute toxicity to fish

For information on studies already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoint from a previously evaluated study is stated in Table 8.2- 1 in grey. In addition, a study on sheepshead minnow has been conducted to fulfil a requirement of USEPA. This study is submitted for the current EU review and is summarised below, and the endpoint is included in Table 8.2- 1.

Report:	KCA 8.2.1/04; [redacted] S.; [redacted]; [redacted]; 2013; M-444829-01-1
Title:	Acute toxicity of ethephon to the sheepshead minnow (Cyprinodon variegatus) under flow-through conditions
Report No.:	EBETL014
Document No.:	M-444829-01-1
Guideline(s):	FIFRA 72-3, OPPTS Guideline 850.1075, OECD Guideline no. 203
Guideline deviation(s):	Routine spring water and reverse osmosis water contaminant screening analyses for pesticides, PCBs and toxic metals were conducted by American Analytical Laboratories, Akron, OH. these data were not collected in accordance with Good Laboratory Practice procedures (no protocol, study director, or in-life inspections).[40CFR160.90(g)]
GLP/GEP:	**yes**

Objective:

To determine the acute toxicity of ethephon to juvenile sheepshead minnow (*Cyprinodon variegatus*).

Material and Methods:

The test item was ethephon 'Base 250' (analysed: 73.8% w/w a.s.) of batch number 03022F913-SA. Fish were exposed under flow-through conditions for 96 hours. There was one replicate of 10 fish for the control and each test concentration. The nominal concentrations were: 0 (control), 6.25, 12.5, 25, 50 and 100 mg a.s./L. Test solutions were analysed for ethephon. Mean measured concentrations ranged from 94 to 118% of nominal. Results are based on the mean measured concentrations.

Results:

Ethephon: Results of an acute toxicity study on sheepshead minnow:

Mean Measured Conc. (mg a.s./L)	Hour 4		24 Hours		48 Hours		72 Hours		96 Hours	
	Dead	Obs	Dead	Obs	Dead	Obs	Dead	Obs	Dead	Obs
0 (control)	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
7.39	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
12.5	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
24	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N
47	0	10 N	0	10 N	0	10 N	0	9N, 1 P*	0	10 N
102	0	10 N	0	10 N	0	10 N	0	10 N	0	10 N

N = Normal, P = Pale, Obs = Observations (number of individuals observed plus observation).
* One fish was pale in colour which was not believed to be treatment-related.

Conclusions:

The 96h-LC_{50} was >102 mg a.s./L. The NOEC was 102 mg a.s./L.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

For information on the study already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoint from this study is stated in Table 8.2- 1 in grey text.

As requested by the RMS, EC_{10} and EC_{20} values should be determined as additional endpoints to the fish ELS study ([redacted], 2001, M-205148-01-2). Results were reevaluated in a separate statistical report, which can be provided on request, and a summary is presented below.

Introduction
A statistical evaluation addressing the calculation of EC_{10} and EC_{20} values was conducted with the results of the study M-205148-01-2 ([redacted], 2001) to fulfill the data requirements according to regulation EU 283/2013.

Statistical evaluation
The study M-205148-01-2 ([redacted], 2001) was statistically evaluated for the effects of ethephon technical on the fish *Pimephales promelas*. The organisms were exposed for 34 days to the following concentrations of ethephon technical: 5.0, 10.0, 21.0, 43.0 and 86.0 mg a.s./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from original study report and corrected to the control response.
The effects on all the parameters used in the study (embryo hatchability and survival, larval survival, length and weight) were used for the statistical evaluation. In order to derive concentrations with an effect of 10 or 20% (EC_{10} and EC_{20}) on the organisms according to the various parameters, several statistical analyses were performed with the software ToxRatPro Version 3.2.1 (ToxRat Solutions GmbH, 2015).

Results
Due to the lack of a concentration response, it was not possible to derive valid EC_{10} and EC_{20} from the results of the study. The software calculated EC_{10} and EC_{20} values for the effect of ethephon technical on the embryo hatchability and survival using a Probit regression analysis, however, according to the statistical parameters presented, p(F) = 0.183; p (Chi^2) <0.001 together with the lack of confidence intervals both values should not be considered valid. Details on the statistical evaluation can be found in the report.

Conclusions
According to the statistical analysis performed, it was not possible to calculate valid EC_{10} and EC_{20} values for any of the parameters evaluated in the considered study.

CA 8.2.2.2 Fish full life cycle test

An early life-stage study (ELS) is already available. Ethephon has a low toxicity in the ELS study, is not persistent in sediment-water systems, has a very low $logK_{ow}$, and shows no indications of any

interactions with endocrine systems. On this basis, it is considered than a fish full life-cycle study is not required.

CA 8.2.2.3 Bioconcentration in fish

The log K_{ow} for ethephon is <3. Hence, a fish bioconcentration study is not required.

CA 8.2.3 Endocrine disrupting properties

Based on the analysis of the complete toxicological data set, there is no evidence of any endocrine disrupting potential of ethephon in mammals. Likewise in studies with birds, fish and other aquatic organisms no indication of an endocrine activity was found. Therefore it is concluded that ethephon has no endocrine disrupting activity in environmental organisms. Further special testing for endocrine disrupting properties is therefore not warranted. Further details are provided in a Position Paper which is included in Appendix 1.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

In the previous EU review, the available acute toxicity study on *Daphnia magna* was concluded to be invalid (the LC_{50} from the *Daphnia* reproduction study of >160 mg a.s./L was used as the official endpoint). A new acute toxicity study has been conducted for the current EU review. The study is summarised below, and the endpoint is stated in Table 8.2- 1.

Report: KCA 8.2.4.1/02; [redacted], 2015; M-524938-01-1
Title: Acute toxicity of ethephon (technical concentrate) to the waterflea Daphnia magna in a static-renewal laboratory test system - Limit test
Report No.: EBEDN025
Document No.: M-524938-01-1
Guideline(s): OECD Test Guideline No. 202; EEC Directive 92/69, Method C2.
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

To determine the influence of the test item on mobility of *Daphnia magna* over 48 hours by static-renewal exposure (test media renewed after 24 h), expressed as the EC_{50} for immobilisation.

Material and Methods:

The test item was ethephon technical concentrate (73.6 % w/w a.s. analysed) of batch no. HR4C21X02. *Daphnia magna* (1st instar < 24 h old, 10 × 5 animals per concentration) were exposed in a static-renewal test system for 48 hours to a single nominal concentration of 100 mg a.s./L (136 mg technical concentrate/L). After 24 and 48 hours, the behaviour was visually evaluated by counting mobile daphnids and recording any sub-lethal effects. Ethephon was analytically quantified in freshly-prepared test media and in aged test media after 24 hours, for both of the media exchanges.

Results:

Measured concentrations were 103-113% of nominal in fresh media and 73.9-77.2% of nominal in the 24 hour aged media. Results were expressed as the geometric mean of measured concentrations in fresh and aged media, which was 90.4 mg a.s./L. No immobilisation nor lethal effects were observed.

Conclusions:

The 48 h EC_{50} for *Daphnia magna* was >90.4 mg a.s./L. The NOEC was 90.4 mg a.s./L.

Report: KCA 8.2.4.1/03; [redacted]; 2011; M-520027-01-1
Title: Evaluation of acute toxicity and teratogenic effects of plant growth regulators by Daphnia magna embryo assay.

Report No.: M-520027-01-1
Document No.: M-520027-01-1
Guideline(s): not applicable
Guideline deviation(s): not applicable
GLP/GEP: **no**

EXECUTIVE SUMMARY

This study evaluated the toxicity of plant growth regulators, including Ethephon, to *Daphnia magna*. The methods used included a traditional neonate acute toxicity test, a new Daphnia embryo toxicity test, and a teratogenic embryo test. In the neonate acute toxicity test, EC_{50} values of 149.7 mg l^{-1} (24h) and 130.5 mg l^{-1} (48h) were found. In the embryo acute toxicity tests, a 48h EC_{50} of 125 mg l^{-1} and a 48h NOEC of 48 mg l^{-1} were found. In the embryo developmental teratogenic assay, an EDI rate (embryo development inhibition) of 15% was found after 48h.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Ethephon
Active substance(s):	Ethephon
Chemical state and description:	CAS No. 16672-87-0
Source of test item:	[redacted], China)

Batch number:	not specified
Purity:	98%
Storage conditions:	No data
Water temperature:	20 ± 2°C
Water solubility:	131mg l^{-1} (20°C)

2. Test organism(s)

Species:	*Daphnia magna*
Source of test species:	[redacted] Taiwan
Culture conditions:	*Daphnia magna* have been maintained parthenogenetically in [redacted] Taiwan since 2001. They have been kept in 10-l tanks on a windowsill of the laboratory at approximately 20 °C. The tanks generated enough green alga (*Chlorella vulgaris*) to sustain a colony of several hundred for at least 6 months. The tanks were topped up alternatively with dechlorinated and conditioned tap water to replenish water lost by evaporation and then aerated with filtered air.

B. Study design and methods

1. Test procedure

Test system:	• 48h Neonate acute toxicity tests • 48h Embryo acute toxicity tests • 72 h Embryo developmental teratogenic assay (extended duration)
Test concentration(s):	not specified
Control(s):	Yes (no indication about the use of solvent)
Replicates:	• Neonate acute toxicity tests: 4 replicates per concentration (5 daphnids per replicate) • Embryo acute toxicity tests: No clear information (probably 4) • Embryo developmental teratogenic assay: No clear information (probably 4)
Repetitions::	• Embryo acute toxicity tests: 4 times. • Embryo developmental teratogenic assay: probably 4 times because data based on embryo acute toxicity tests
Test conditions:	• high hardness medium (COMBO medium) • Neonate acute toxicity tests: 20±2°C, 16/8-h light/dark cycle • Embryo acute toxicity tests: 20±2°C, 16/8-h light/dark cycle • Embryo developmental teratogenic assay: probably 20±2°C, 16/8-h light/dark cycle because data based on embryo acute toxicity tests • Test vessels: 50 ml of medium in 100 ml glass beakers
Feeding:	• Not specified
Medium renewal:	• Not specified
Frequency of test item application:	• Not specified
Test duration:	• See “Test system” above
Endpoints:	• Neonate acute toxicity tests: 24h and 48h EC_{50} – Immobility (daphnids showing no movement within 15 s after gentle stirring were defined to be immobile) Toxicity ratio: Neonate 24 h EC_{50}/Neonate 48 h EC_{50}

- Embryo acute toxicity tests: 48h EC_{50} and 48h LOEC
  Toxicity ratio: Neonate 48 h EC_{50}/Embryo 48 h EC_{50}
  Toxicity ratio: Neonate 48 h EC_{50}/Embryo 48 h LOEC
- Embryo developmental teratogenic assay: 24, 48 and 72h LOEC – abnormality rate, EDI (embryo development inhibition rate), investigated organs: second antennae, rostrum, malpighian tube, sensory bristles, tail spine

Statistics:

- Neonate acute toxicity tests: The EC_{50} values were calculated by probit analysis based on nominal concentrations.
- Embryo acute toxicity tests: The 48 h EC_{50} values and 95% confidence limits were calculated by probit analysis on the basis of nominal concentrations.
- Embryo developmental teratogenic assay: Not specified

RESULTS

1. Validity criteria:

- Neonate acute toxicity tests: No mortality in the control group.
- Embryo acute toxicity tests: No mortality or growth retardation in the control group.
- Embryo developmental teratogenic assay: Not specified

2. Biological findings:

Table 1: Toxicity values of Ethephon on *D. magna* neonates after 24 h and 48 h exposure (n = 20).

Plant growth regulator (mg l^{-1})	24 h EC_{50}	48 h EC_{50}	24 h EC_{50}/ 48 h EC_{50}
Ethephon	149.7 ± 7.1	130.5 ± 3.2	1.1

Table 2: Toxicity values of Ethephon on *D. magna* embryos after 48 h exposure (n = 20).

Plant growth regulator (mg l^{-1})	Neonate 48 h EC_{50}	Embryo 48 h EC_{50}	Embryo 48 h LOEC	Neonate 48 h EC_{50}/ Embryo 48 h EC_{50}	Neonate 48 h EC_{50}/ Embryo 48 h LOEC
Ethephon	130.5 ± 3.2	125 ± 6.3	48 ± 2.3	1.1	2.7

Table 3: Comparison of LOEC abnormality rate of *D. magna* embryos caused by Ethephon after 24 h, 48 h, and 72 h exposure (n = 20).

D. magna organs	Ethephon			
	24	48	72	48/72
Second antennae	0	25	25	100
Rostrum	0	0	0	-
Malpighian tube	0	20	50	40
Sensory bristles	0	10	15	67
Tail spine	0	10	25	40
EDI rate	0	15	DG	ND

EDI: embryo development inhibition, DG: deformed growth, ND: no detection.

RESULTS SUMMARY

In the neonate acute toxicity test, EC_{50} values of 149.7 mg l^{-1} (24h) and 130.5 mg l^{-1} (48h) were

found. In the Embryo acute toxicity tests, a 48h EC_{50} of 125 mg l^{-1} and a 48h LOEC of 48 mg l^{-1} were found. In the Embryo developmental teratogenic assay, an EDI rate (embryo development inhibition) of 15% was found after 48h. The toxicity ratios of neonate 24 h EC_{50}/neonate 48 h EC_{50} and neonate 48 h EC_{50}/embryo 48 h EC_{50} are 1.1 while the toxic ratio of neonate 48 h EC_{50}/embryo 48 h LOEC is 2.7. This indicates that ethephon is slightly more toxic to embryos than to neonates.

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CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

An acute toxicity study on Eastern oyster (*Crassostrea virginica*), a marine species, was conducted in 1989 in order to satisfy a US EPA requirement. The study is submitted now for completeness. The study is summarised below and the endpoint is included in Table 8.2-1.

Report:	KCA 8.2.4.2/01; [redacted]; 1989; M-187969-01-1
Title:	(Ethephon) - Acute Toxicity to Eastern Oyster (Crassostrea virginica) under Flow-Through Conditions
Report No.:	R013450
Document No.:	M-187969-01-1
Guideline(s):	US EPA FIFRA Guideline 72-3 (1985)
Guideline deviation(s):	--
GLP/GEP:	**yes**

Objective:

To determine the acute toxicity of ethephon to Eastern oyster under flow-through conditions in a 96-hour toxicity test, expressed as EC_{50} and NOEC for shell deposition.

Material and Methods:

The test item was ethephon technical concentrate (analysed: 72.2 % w/w a.s.) of batch no. 4022193. *Crassostrea virginica* (~1 year old, mean valve height of 37 ±4 mm) were exposed in a flow-through test system for 96 hours to nominal concentrations of 0 (control), 19, 32, 54, 90 and 150 mg a.s./ L in natural unfiltered seawater. Forty oysters were exposed in duplicate test aquaria (20 per aquaria) per treatment. The concentration of ethephon in exposure media was measured at start and end of the exposure period. Test water had a salinity of 32‰, a pH of 8.0 to 8.1 and a temperature of 20 ± 2 °C. Photoperiod was maintained at 16 hours light and 8 hours of dark. Observations were made daily to detect mortality or any abnormalities. After 96 hours, oysters were removed from test aquaria and new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer. Effect concentration and confidence intervals which resulted in 50% reduction of shell deposition was calculated by probit transformation of the growth data (expressed as percent reduction) and log transformation of the concentration, followed by the method of Inverse prediction. The NOEC was determined by using Williams test coupled with Bartlett's test for determination of homogeneity of variances or the Kruskal-Wallis test if homogeneity of variances could not be confirmed.

Results:

The chemical analysis of ethephon on days 0 and 4 resulted in mean measured concentrations of 17, 30, 47, 84 and 150 mg a.s./L.

At test termination, no mortality was observed in the control or any treatment group tested.

Results of a toxicity study with ethephon on *Crassostrea virginica*:

Mean measured concentration (mg a.s./L)	Exposed oysters (n)	Mean shell deposition (Standard deviation) in mm	Mean percentage reduction[a]
0 (control)	40	2.5 (1.0)	NA
17	40	2.0 (0.9)	20
30	40	1.7 (0.7)	32
47	40	1.4 (0.8)	44
84	40	0.7 (0.5)	72
150	40	0 (0)	100

[a] % reduction in shell growth as compared to the shell growth of the control oysters.

Conclusions:

The 96 hour EC_{50} for reduction of shell growth of Eastern oyster *(Crassostrea virginica)* was 60 (25 – 93) mg a.s./L and the NOEC was <17 mg a.s./L (the lowest concentration tested).

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

No additional studies have been performed. For information on the study already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoints from this study are stated in Table 8.2- 1 in grey text.

As requested by the RMS, EC_{10} and EC_{20} values should be determined as additional endpoints to the chronic toxicity study to *Daphnia magna* ([redacted], 1992, M-187833-01-1). Results were reevaluated in a separate statistical report, which can be provided on request, and a summary is presented below.

Introduction

A statistical evaluation addressing the calculation of EC_{10} and EC_{20} values was conducted with the results of the study M-187833-01-1 ([redacted], 1992) to fulfill the data requirements according to regulation EU 283/2013.

Statistical evaluation

The study M-187833-01-1 ([redacted], 1992) was statistically evaluated for the effects of Ethephon technical on the aquatic invertebrate *Daphnia magna*. The organisms were exposed for 21 days to the following concentrations of ethephon technical: 8.5, 17.0, 38.0, 67.0 and 160.0 mg a.s./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from original study report. In the original study report, the calculated NOEC was 67 mg a.s./L.

In order to derive Effect Concentrations that have 10 and 20 % effects on the number of juveniles per introduced parent of the test subjects (EC_{10} and EC_{20}), a Normal Sigmoid (3 parameters) non-linear regression analysis was performed with the software ToxRatPro Version 3.2.1 (ToxRat Solutions GmbH, 2015). To obtain more precise and reliable results, the number of optimizing cycles was doubled to 1000.

Results

According to the statistical parameters; F (2, 21) = 6.524; p(F) = 0.006; R2 = 0.383 the EC_{10} and EC_{20} calculated for the number of offspring per introduced parent, values should be considered valid. After non-linear regression no lack of fit was detected for the function (p[F|Lack of Fit) = 0.155.

The obtained EC_{10} and EC_{20} values are presented in the table below.

Results of the normal sigmoid 3 parameters non-linear regression analysis with the cumulative offspring per introduced parent of the introduced *Daphnia magna* at day 21: Selected effective concentrations (ECx) of the test item and their 95%-confidence limits (according to Fieller's theorem).

Toxicity	EC_{10} (95 % confidence interval) [mg a.s./L]	EC_{20} (95 % confidence interval) [mg a.s./L]
Effect on number of offspring per introduced parent	122.676 (31.297 - 480.855)	151.111 (32.317-714.020)

Conclusions

The calculated EC_{10} and EC_{20} values are 122.676 and 151.111 mg a.s./L, respectively. The statistical parameters presented showed that these values can be considered valid.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

No chronic studies on additional aquatic invertebrate species are required since ethephon is not an insecticide and does not show an insecticidal mode of action.

CA 8.2.5.3 Development and emergence in *Chironomus* species

Ethephon does not have any insecticidal properties. Hence, a study on development and emergence of *Chironomus* species is not required.

CA 8.2.5.4 Sediment dwelling organisms

Ethephon does not have any insecticidal properties. Hence, a study on development and emergence of *Chironomus* species is not required.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

For information on studies already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoints from these previously-evaluated studies are stated in Table 8.2- 1 in grey text. In addition, a new study on the freshwater diatom, *Navicula* has been conducted. This was done to provide a study on an additional green algal species which is fully compliant with OECD Guideline No. 201 (2006). For completeness, an existing study on the marine alga *Skeletonema* is also submitted (study was conducted to satisfy USEPA requirements). Both these additional studies are summarised below, and their endpoints are included in Table 8.2- 1.

Report:	KCA 8.2.6.1/05; [redacted]; 2015; M-534339-01-1
Title:	Toxicity of ethephon technical to the freshwater diatom Navicula pelliculosa during a 96 hour exposure
Report No.:	007SRUS15C1-10
Document No.:	M-534339-01-1
Guideline(s):	OECD Guideline No. 201 (2006)
Guideline deviation(s):	The afore-mentioned guidelines were harmonized for various test parameters (i.e. temperature, light, etc.) to achieve optimal environmental conditions for the test organism. Scientific discretion was implemented where guideline parameters do not fully converge.
GLP/GEP:	**yes**

Objective:

The objective was to determine the effect of ethephon on the growth of *Navicula pelliculosa*.

Material and Methods:

The test item was ethephon technical concentrate (73.6 % w/w a.s.) from batch no. HR4C21X02. *N. pelliculosa* was exposed for 96 hours under static conditions to nominal concentrations of 0.625, 1.25, 2.50, 5.00 and 10.0 mg a.s./L. There was a water and solvent control (N,N-dimethylformamide). There were four replicate vessels per test level and control. The initial cell number was 10,000 cells/mL. Growth inhibition was calculated using algal biomass per volume. pH values in the controls ranged from 7.4 to 7.5 at test initiation, at test termination the pH was 9.5 to 9.6. Temperature ranged from 23.6 to 24.1°C at an illumination of 4470 to 4860 lux. Concentrations of ethephon in test media were analysed on day 0, day 3 and day 4. Growth rate was based on change in cell density from day 0 to day 3 and day 0 to day 4. Cell density was determined by manual counts via light microscope and hemocytometer slide. Statistical analysis of data from control and solvent control were compared to

evaluate if they could be pooled. For determining endpoints data were first checked for normality (Shapiro-Wilks test) and homogeneity of variance (Bartlett equality of variance). The NOEC was calculated by analysis of variances (ANOVA) followed by a Dunnett's test. EC_{10}, EC_{20} and EC_{50} values were determined. Statistical analyses were conducted with CETIS v.1.8.7.4.

Results:

Results of analytical determinations of ethephon in test media:

Nominal Conc. (mg a.s./L)	Day 0	Day 3 (72 h)		Day 4 (96 h)	
	Measured Conc. (mg a.s./L)	Measured Conc. (mg a.s./L)	Geometric Mean Measured Conc. (mg a.s./L)	Measured Conc. (mg a.s./L)	Arithmetic Mean Measured Conc. (mg a.s./L)
Control	<LOQ	< LOQ	NA	< LOQ	NA
S. Control	<LOQ	< LOQ	NA	< LOQ	NA
0.625	0.522	0.0615	**0.179**	0.0230	**0.203**
1.25	1.098	0.143	**0.396**	0.0651	**0.435**
2.5	2.06	0.245	**0.711**	0.113	**0.807**
5	4.26	0.519	**1.49**	0.213	**1.66**
10	8.16	1.00	**2.86**	0.413	**3.19**

Limit of quantification (LOQ) = 0.05 mg a.s./L, NA = Not Applicable

Initial measured concentrations were close to nominal. This was followed by substantial decline as determined on Day 3. Hence, biological results are based on mean measured concentrations. No cell abnormalities were observed in the control and treatment groups.

Effect of ethephon on the freshwater diatom *Navicula pelliculosa* in a 96 h growth inhibition test:

Nominal concentration [mg a.s./L]	Day 1 (24 h)	Day 2 (48 h)	Day 3 (72 h)	Day 4 (96 h)
	Mean cell number x 10^4 per mL	Mean cell number x 10^4 per mL	Mean cell number x 10^4 per mL	Mean cell number x 10^4 per mL
Control	3.25	41.28	131.75	340.50
Solvent Control	3.19	41.16	130.06	340.50
0.625	3.17	40.16	129.94	338.75
1.25	3.01	31.10	119.81	327.25
2.50	2.99	25.92	119.50	330.25
5.00	3.15	23.94	121.56	318.00
10.00	2.88	19.96	84.50	294.75

Test initiation with 10,000 cells/mL

Control and solvent control were not significantly different ($p \leq 0.05$). Therefore, controls were pooled for statistical evaluation.

Endpoints for ethephon on *Navicula pelliculosa* in a 96 h test based on mean measured concentrations:

Endpoint	72 hours	96 hours
E_rC_{50}	>2.86 mg a.s./L	>3.19 mg a.s./L
LOEC	2.86 mg a.s./L	1.66 mg a.s./L
NOEC	1.49 mg a.s./L	0.807 mg a.s./L

Conclusions:

The 72-h growth rate was based on geometric mean measured concentrations from days 0 and 3. The 72-h E_rC_{50} is >2.86 mg a.s./L with a LOEC and NOEC of 2.86 and 1.49 mg a.s./L, respectively. The 96-h growth rate was based on mean measured concentrations from days 0, 3 and 4. The 96-h E_rC_{50} is >3.19 mg a.s./L with a LOEC and NOEC of 1.66 and 0.807 mg a.s./L, respectively.

Report:	KCA 8.2.6.1/06; [redacted]; 1990; M-187843-01-1
Title:	Ethephon - Toxicity to the Marine Diatom Skeletonema Costatum
Report No.:	R013382
Document No.:	M-187843-01-1
Guideline(s):	USEPA FIFRA §122-2 and §123-2 (1982)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To determine the effect of ethephon on the growth of the marine diatom *Skeletonema costatum.*

Material and Methods:

The test item was ethephon 'Base 250' (containing 71.9 % w/w a.s.). *S. costatum* was exposed for 120 hours under static conditions to the mean measured concentration of 1.8 mg a.s./L in comparison to a control group. There were three replicate vessels per test level and control. The initial cell number was 10,000 cells/mL. Growth inhibition was calculated using algae biomass per volume. pH in the controls ranged from 8.0 to 8.1 at test initiation, at test termination the pH was 9.2. The temperature ranged from 19 to 21°C at an illumination of 4000 to 5000 lux. Concentrations of ethephon were quantified on day 0 and day 3 of the exposure period.

Results:

The average cell densities of the three replicates exposed to ethephon and of the three control replicates were calculated for each observation period (24, 48, 72, 96 and 120 h), and the mean cell density of the exposed cultures was expressed as a percentage of the mean cell density of control cultures. The measured concentration of test solutions for ethephon at test initiation was 1.8 mg a.s./L (122% of nominal). At test termination, 0.18 mg a.s./L (12% of nominal) remained in the test solution. The likely cause of the decline was hydrolysis.

Effect of ethephon on marine diatom (*Skeletonema costatum*) in a 120 h growth inhibition test:

Initial mean measured concentration [mg a.s./L]	Day 3 (72 h)	Day 5 (120 h)	
	Mean cell number x 10^4 per mL	Mean cell number x 10^4 per mL	Inhibition
Control	111.92	285.83	n.a.
1.8	138.58	280.50	2%

Test initiated with 10,000 cells/mL

Cell densities increased over time in all replicates. Mean cell densities in the replicates exposed to an initial 1.8 mg a.s./L were 90%, 106%, 124%, 100% and 98% of mean cell densities of controls at 24, 48, 72, 96 and 120 hours, respectively.

Conclusions:

Mean cell density in cultures exposed to an initial measured concentration of 1.8 mg a.s./L was 98% of the mean cell density in control. Therefore, the EC_{50} was >1.8 mg a.s./L.

CA 8.2.6.2 Effects on growth of an additional algal species

For information on the *Anabaena* study evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoint from this study is included in Table 8.2- 1 in grey text.

CA 8.2.7 Effects on aquatic macrophytes

For information on the *Lemna* study evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The endpoint from this study is included in Table 8.2- 1 in grey text.

As requested by the RMS, E_yC_{50} and E_rC_{50} values and corresponding NOEC and LOEC values should be determined as additional endpoints to the study on *Lemna* ([redacted], 1990, M-187845-01-1). Results were reevaluated in a separate statistical report and can be provided on request. A summary is presented below.

Introduction

A statistical evaluation addressing the calculation of EC_{50} for yield and growth rate was conducted with the result data of the study M-187845-01-1 ([redacted], 1990) to fulfill the data requirements according to regulation EU 284 and 283/2013.

Statistical evaluation

The study M-187845-01-1 ([redacted] 1990) was statistically evaluated for the effects of Ethephon on the freshwater plant Lemna gibba. The organisms were exposed for 14 days to the following concentrations of ethephon: 0.10, 0.17, 0.45, 0.88 and 1.6 mg a.i./L. Additionally, a control was tested in parallel. The data used for this evaluation were obtained from original study report.
In order to derive Effect Concentrations causing 50 % effects on yield and growth rate of the test subjects (EC_{50}), a 3 param. normal CDF non-linear regression analysis was performed with the software ToxRatPro Version 3.2.1.

Results

The obtained EC_{50} value for yield is above the tested range of test concentrations (i.e. greater than 1.6 mg a.s./L; F (2, 21) = 60.257; p(F) < 0.001; R2 = 0.885; see Appendix 1) and therefore considered invalid.
An EC_{50} value for growth rate cannot be calculated due to mathematical reasons and a lack of dose-response relationship.

Conclusions

The obtained EC_{50} value for yield is greater than the maximum test concentration of 1.6 mg a.s./L and cannot be considered reliable.
A EC_{50} value for growth rate cannot be calculated due to mathematical reasons

In addition, a growth inhibition study on *Myriophyllum spicatum* has been conducted for the current EU review. This study is summarised below, and the endpoint is stated in Table 8.2- 1.

Report: KCA 8.2.7.02; [redacted]; 2015; M-537257-01-1
Title: Toxicity of ethephon (technical concentrate) to the aquatic plant Myriophyllum spicatum in a semi-static growth inhibition test
Report No.: EBF EN042
Document No.: M-537257-01-1
Guideline(s): OECD Guideline No. 239 Water-sediment Myriophyllum spicatum toxicity test.
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The objective of the study was to determine the effect of ethephon on the vegetative growth of the freshwater aquatic plant *Myriophyllum spicatum*.

Material and Methods:

The test item was ethephon technical concentrate (73.6 % w/w a.s. analysed) of batch no. HR4C21X02. This study included seven treatment groups (nominal: 0.298, 0.954, 3.05, 9.77, 31.3 and 100 mg a.s./L; and a control) with four replicates per test concentration and six replicates for the control. After an establishment phase of 7 days, 3 plants per replicate were exposed for 14 days under semi-static conditions in the presence of sediment. Test medium was replaced on day 3, 7 and 10. The light regime was 16 h light and 8 h dark. Light intensity was 150 µE·m^{-2} s^{-1} (mean value) with a range of 148 - 158 µE·m^{-2} s^{-1}. The total shoot length was determined at test start, on day 3, 7, 10 and 14. On day 14, the fresh and dry weight of each plant was determined. The samples collected at test start, at each water exchange and after 14 days were analysed via LC-MS/MS method. Water temperature was 19.5-21.6 °C. pH in freshly prepared media was 6.3-7.9, pH in aged media was 6.3-9.2.

Results:

In the freshly prepared test media 77 to 97 % of the nominal test concentration was found (average of all test concentrations). In the aged test media, 17 to 62 % of the nominal value was determined (average of all test concentrations). Since the ethephon concentrations decreased within the water exchange intervals, the time weighted mean measured concentration was calculated in addition for each treatment group. Time-weighted mean measured concentrations of were 0.152, 0.489, 1.65, 5.51, 15.8 and 70.7 mg a.s./L. The effect on shoot length, fresh weight and dry weight and the results of the visual assessments of plants and roots are presented on the following pages.

***Myriophyllum spicatum*: Growth rates µ (based on total shoot length) and percentage inhibition of µ (based on total shoot length) after 14 days of exposure (test end):**

Test concentration	Growth rate µ [1/day] after 14 days						
[mg a.s./L]	Control	0.298	0.954	3.05	9.77	31.3	100
Replicate							
1	0.049	0.059	0.050	0.065	0.065	0.069	0.071
2	0.043	0.054	0.052	0.046	0.052	0.086	0.070
3	0.059	0.056	0.057	0.042	0.071	0.076	0.093
4	0.056	0.061	0.064	0.055	0.064	0.077	0.089
5	0.053						
6	0.050						
m	**0.052**	**0.058**	**0.056**	**0.052**	**0.063**	**0.077**	**0.081**
s	0.006	0.003	0.006	0.010	0.008	0.007	0.012
% inhibition	**-**	**-11.3**	**-7.9**	**-0.6**	**-21.9**	**-49.0**	**-56.3**

- % inhibition: increase in growth relative to that of control

* mean value significantly different from the control (tested with Williams t-test, α = 0.05, one-sided)

m: mean value

s: standard deviation

***Myriophyllum spicatum*: Growth rates µ (based on fresh weight) and percentage inhibition of µ (based on fresh weight) after 14 days of exposure (test end):**

Test concentration [mg a.s./L]	Growth rate µ [1/day] fresh weight						
	Control	0.298	0.954	3.05	9.77	31.3	100
Replicate							
1	0.053	0.070	0.050	0.071	0.069	0.072	0.021
2	0.034	0.067	0.055	0.058	0.067	0.071	0.048
3	0.049	0.053	0.071	0.040	0.062	0.116	0.075
4	0.050	0.097	0.085	0.057	0.068	0.093	0.070
5	0.065						
6	0.066						
m	**0.053**	**0.072**	**0.065**	**0.057**	**0.069**	**0.088**	**0.054**
s	0.012	0.018	0.016	0.013	0.014	0.021	0.025
% inhibition	-	**-35.8**	**-23.5**	**-6.9**	**-30.6**	**-66.6**	**-1.3**

- % inhibition: increase in growth relative to that of control

* mean value significantly different from the control (tested with Williams t-test, α = 0.05, one-sided)

m: mean value

s: standard deviation

***Myriophyllum spicatum*: Growth rates µ (based on dry weight) and percentage inhibition of µ (based on dry weight) after 14 days of exposure (test end):**

Test concentration [mg a.s./L]	Growth rate µ [1/day] dry weight						
	Control	0.298	0.954	3.05	9.77	31.3	100
Replicate							
1	0.020	0.037	0.015	0.028	0.058	0.044	-0.006
2	0.001	0.036	0.015	0.024	0.031	0.042	0.016
3	0.017	0.015	0.036	0.006	0.033	0.072	0.032
4	0.016	0.054	0.052	0.017	0.026	0.056	0.039
5	0.034						
6	0.030						
m	**0.020**	**0.036**	**0.030**	**0.019**	**0.037**	**0.054**	**0.020**
s	0.012	0.016	0.018	0.010	0.014	0.014	0.020
% inhibition	-	**-80.5**	**-50.0**	**4.7**	**-88.1**	**-172.0**	**-3.0**

- % inhibition: increase in growth relative to that of control

* mean value significantly different from the control (tested with Williams t-test, α = 0.05, one-sided)

m: mean value

s: standard deviation

Myriophyllum spicatum: **Assessment of plant health:**

Test concentration [mg a.s./L]	Sublethal Effects during Exposure					
	0.298	0.954	3.05	9.77	31.3	100
Exposure time						
Day 0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0
Day 7	0	0	0	0	0	0
Day 10	0	0	0	0	0	0
Day 14	0	0	0	0	0	9 (12)

1: weaker plants
2: leaves laid to the stem (loss of turgor)
3: necrosis
4: chlorosis
5: rose shoot tips
6: white shoot tips
7: shortened shoot tips
8: thickened nodes
9: shoot tip deformation (slight)
Values in parentheses indicate the number of plants where the effects were observed

Myriophyllum spicatum: **Assessment of root health (compared to the control performance):**

Test concentration [mg a.s./L]	Root development after 14 days (test end)
0.298	1
0.954	1
3.05	1
9.77	1
31.3	1
100	1

1: healthy roots, comparable to the control
2: shortened roots
3: only few roots
4: weaker roots
5: no roots

The mean total shoot length and mean total shoot fresh weight in control plants increased by a factor of 2.1 within the exposure phase of the test. Therefore this validity criterion was met. The control plants did not show any signs of chlorosis. A thin algal layer between sediment and sand was seen at the test end in all test vessels. Since the test design is not sterile this algal growth could not be avoided. Since this algal contamination was only minor and occurred in all test vessels the test is considered to

be valid. The mean coefficient of variation in yield based on measurements of shoot fresh weight (i.e. from test initiation to test end) in control cultures was 30.1 Therefore, this validity criterion was met.

Although the growth in the controls met the validity criterion, it can still be considered that the extent of growth in the control over the 14 day period was relatively low. The higher growth than the control in the treatments should therefore be treated with caution since relatively small differences between controls and treatments (which is the case in this current study) tend to be exaggerated in this situation. Endpoints from the study are stated in the table below.

***Myriophyllum spicatum*: Endpoints from a study on ethephon:**

Parameter	Growth rate (total shoot length) [mg a.s./L]	Growth rate (fresh weight) [mg a.s./L]	Growth rate (dry weight) [mg a.s./L]
EC_{50} (14 day)	> 100 (> 70.7)	> 100 (> 70.7)	> 100 (> 70.7)
14 day NOEC	100 (70.7)	100 (70.7)	100 (70.7)
14 day LOEC	> 100 (> 70.7)	> 100 (> 70.7)	> 100 (> 70.7)

Conclusions:

No adverse effects on total shoot length, fresh or dry weight were observed. Therefore, all 14 day E_rC_{50} values were >100 mg a.s./L (nominal) or >70.7 mg a.s./L (time-weighted average of measured concentrations). The 14 day NOEC was 100 mg a.s./L (nominal) or 70.7 mg a.s./L (time-weighted average of measured concentrations). At the nominal 100 mg a.s./L treatment level, measured concentrations in fresh media on day 0, 3, and 10 ranged from 81 to 88% of nominal. Hence, the endpoints can be expressed in terms of the nominal concentration.

Additional information from the Notifier:

Testing on *Myriophyllum spicatum* is particularly challenging in terms being able to achieve OECD Guideline validity criteria. In this case, in order to achieve a coefficient of variation (cv) in the control group which was lower than the validity criterion of 35%, the in-life phase had to be run twice. The results in the study report are for the second running of the in-life phase, which did satisfy the validity criteria. In the first running of the in-life phase the cv in the control for fresh weight yield was 78%. i.e. this growth parameter was clearly invalid. Nevertheless, growth in the control in terms of total shoot length (TSL) was still valid. The extent of growth in the control as TSL from day 0 to day 14 was x3.8, which was more growth than in the second running of the in-life phase. Results for the measurements of TSL at study start and at study termination of the first running are presented below.

Results for the measurement of total shoot length from the first running of the in-life phase:

Treatment [mg a.s./L]	Mean TSL Day 0	Std. Dev.	Mean TSL Day 14	Std. Dev.
Control	8.53	1.051	32.64	4.130
0.298	7.83	1.705	25.46	5.580
0.954	8.92	1.198	31.58	8.888
3.050	8.92	1.198	29.92	1.724
9.770	8.04	1.455	25.38	5.580
31.30	8.42	2.196	28.25	4.029
100.0	9.29	1.350	39.50	2.646

The above results are presented *for information only*, to provide additional context on the apparent 'growth promotion' seen in the second running of the in-life phase. There is no analytical chemistry for the first running and the data have not been subjected to QA review. Hence, they should be treated with caution. Nevertheless, it can be seen above that final TSLs in all treatment levels in the first running were within ±30% of the control. The results illustrate that there can be inherent variability between *Myriophyllum* assays. Hence, in the terms of the second (valid) running of the in-life phase it is not appropriate to interpret the apparent increased growth in the controls as treatment-related.

CA 8.2.8 Further testing on aquatic organisms

Ethephon does not raise concerns in the standard risk assessment. Hence, further testing (such as on additional species in the laboratory or higher-tier studies in outdoor microcosms) is not required.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

For information on studies already evaluated during the previous EU review please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. Previously-evaluated data indicate that ethephon has a low acute oral and contact toxicity to bees (LD_{50} >100 µg a.s./bee). Also, data on other non-target arthropods (CA 8.3.2) do not show any insecticidal activity for ethephon. For completeness, several additional studies have been conducted for the current EU review, in order to fulfil the data requirements under Regulation 1107/2009 (Ref: Data requirements for active substances Regulation 283/2013, dated 1st March 2013). In accordance with Point 4 on page 54 of these data requirements, where appropriate, the tested material in these new studies was the representative plant protection product (Ethephon SL 480). The new studies are summarised later in this section, except where it is stated below that the summary can be found in the MCP. The following bullet points provide the rationale for conducting each study:

- Acute oral and contact toxicity of ethephon to honey bee ([redacted], 2015): Routine study conducted to confirm the results of the study evaluated during the previous EU review.

- Acute oral and contact toxicity of Ethephon SL 480 to honey bee ([redacted], 2014): Routine study, conducted for completeness. Summary is in MCP 10.3.1.1.

- Acute oral toxicity of ethephon to bumble bee ([redacted], 2015a): Study conducted to provide data on an additional bee species.

- Acute contact toxicity of ethephon to bumble bee ([redacted], 2015b): Study conducted to provide data on an additional bee species.

- Chronic toxicity of Ethephon SL 480 to adult honeybee via feeding ([redacted], 2015): Study conducted to fulfil active substance datapoint 8.3.1.2.

- Acute toxicity of ethephon to honey bee larvae ([redacted], 2015): Study conducted to fulfil active substance datapoint 8.3.1.3.

- Honey bee brood (colony) feeding study using Ethephon SL 480 ([redacted], 2015): To fulfil datapoint 8.3.1.3. After study finalisation, it was realised that the sucrose solution containing 2.4 g a.s./L should have been pH-buffered. The pH of a 2.4 g a.s./L aqueous solution of Ethephon SL 480 is 2.0 ([redacted], 2015, M-542286-01-1, KCA 8.3.1.3.03). Uptake of 1 L of treated sucrose solution by each colony was clearly slower than uptake of untreated sucrose solution by control colonies. This was probably related to acidity. The possibility of consequent experimental artefacts could not be excluded. Hence, the study was concluded as unreliable. Subsequently, to replace the study, an acute larval toxicity study ([redacted] 2015) and a honeybee tunnel test ([redacted] 2015) were done.

- Honey bee brood tunnel test using Ethephon SL 480 in which flowering *Phacelia* was sprayed during bee-flight ([redacted] 2015): Conducted to fulfil active substance datapoint 8.3.1.3 and PPP datapoint 10.3.1.5, to provide data on the response of foragers, and brood & colony development. This study is summarised in MCP 10.3.1.5.

Endpoints from studies on bees are presented in Table 8.3.1- 1. The endpoints from the study evaluated in the previous EU review are stated in grey text to distinguish them from endpoints derived from the new studies, which are stated in black.

Table 8.3.1- 1: Ethephon: Endpoints from toxicity studies on bees

Test substance	Study type	Endpoint	References
Ethephon	Honey bee, 48 h	Oral. LC_{50} > 116.5 µg a.s./bee Contact: LC_{50} > 100 µg a.s./bee	LoEP KCA 8.3.1.1.1/01 M-172533-01-2
Ethephon	Honey bee, 48h	Oral. LD_{50} > 111.0 µg a.s./bee Contact: LD_{50} > 100.0 µg a.s./bee	[redacted] (2015) KCA 8.3.1.1.1/02 M-514214-01-1
Ethephon	Bumble bee, 48 h	Oral: LD_{50} > 167.0 µg a.s./bee	[redacted] (2015a) KCA 8.3.1.1.1/03 M-534551-01-1
Ethephon	Bumble bee, 48 h	Contact: LD_{50} > 100.0 µg a.s./bee	[redacted] (2015b) KCA 8.3.1.1.1/04 M-525423-01-1
Ethephon SL 480	Honey bee, 10 days	LDD_{50}: >95.53 µg a.s./bee/day NOEDD: 95.53 µg a.s./bee/day	[redacted] (2015) KCA 8.3.1.2/01 KCP 10.3.1.2/01 M-534554-01-1
Ethephon SL 480	Honey bee brood feeding study	3 colonies each fed 1 L sucrose sol. containing 2.4 g a.s./L. Due to oversight, dosing solution was not pH-buffered. Uptake slower in test item colonies than control, probably due to acidity (pH: 2.0). BTR higher for test item than control. Study is unreliable*.	[redacted] (2015) KCA 8.3.1.3/01 KCA 10.3.1.3/01 M-528291-01-1
Ethephon	Honey bee larvae, acute, 7 days	LD50: >100 µg a.s./larva NOED: 100 µg a.s. /larva	[redacted] (2015) KCA 8.3.1.3/02 M-540682-01-1
Ethephon SL 480	Honey bee, 48h	Oral: LD_{50} >110.7 µg a.s./bee Contact: LD_{50} >100 µg a.s./bee	[redacted] (2014) KCP 10.3.1.1/01 M-504112-01-1
Ethephon SL 480	Honey bee tunnel test, OECD Guidance Document No. 75	No effects on adults, brood or colonies for sprays of 120 & 480 g a.s./ha to flowering *Phacelia* during bee flight. Highest measured residues in pollen & nectar from foragers were 28 and 3 mg a.s./kg, respectively (day 0). Subsequent samples from foragers & combs indicated a rapid decline in concentrations.	[redacted] (2015) KCP 10.3.1.5/01 M-540667-01-1

*Study not suitable for use in risk assessment. To replace this study an acute larval toxicity study ([redacted], 2015) and a honey bee tunnel test assessing brood ([redacted], 2015) were subsequently conducted.
BTR: Brood Termination Rate.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

For information on the study already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The oral toxicity endpoint from this study is included in Table 8.3.1- 1 in grey text. Summaries of new studies on acute oral toxicity to bees are presented below and the endpoints are listed in Table 8.3.1- 1.

Report:	KCA 8.3.1.1.1/02; [redacted]; 2015; M-514214-01-1
Title:	Effects of ethephon tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	92031035
Document No.:	M-514214-01-1
Guideline(s):	OECD Guidelines No. 213 and No. 214 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To determine the acute contact and oral toxicity of ethephon to the honey bee (*A. mellifera* L.).

Material and Methods:

The test item was ethephon technical concentrate (73.6 % w/w a.s.) of batch no. HR4C21X02. Under laboratory conditions 50 worker bees were exposed to a single dose of 100 µg a.s./bee by topical application, and as a control, 50 worker bees were exposed to an equivalent volume of water (+ 0.5% Adhäsit) (contact limit test). Also, 50 worker bees were exposed to a single dose of nominal 100 µg a.s./bee by feeding (in 50% w/v sucrose solution), and as a control, 50 worker bees were exposed to untreated 50% w/v sucrose solution (oral limit test). In terms of actual measured intake in the latter, the dose was 111 µg a.s./bee. Bees were observed during the 48 h after dosing.

Results:

Contact Test: By 48 hours after dosing, 10.0 % mortality had occurred in the 100 µg a.s./bee group and in the control group. No behavioural effects were observed.

Oral Test: The actual measured intake was 111 µg a.s./bee. By 48 hours after dosing, there was 2% mortality. In the control group no mortality occurred. No behavioural effects were observed.

Ethephon: Acute toxicity to honey bees in laboratory tests

Exposure route	contact	oral
Dose (µg a.s./bee)	100.0	111.0
LD_{50} (µg a.s./bee)	> 100.0	> 111.0
NOED (µg a.s./bee)	100.0	111.0

Validity criteria:

Mortality of honey bees in the control (contact test):	10 % (required: ≤ 10%)
Mortality of honey bees in the control (oral test):	0 % (required: ≤ 10%)
LD_{50} of Reference Item (24 hrs), Contact test:	0.18 µg a.s./ bee (required: 0.10-0.30 µg a.s./ bee)
LD_{50} of Reference Item (24 hrs), Oral test:	0.13 µg a.s./ bee (required: 0.10 - 0.35 µg a.s./bee)

The contact and oral tests are considered valid as the control mortality was ≤ 10% and the LD_{50} values for the reference item (dimethoate) were within the required ranges.

Conclusions:

The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 111.0 µg a.s./bee.

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Report:	KCA 8.3.1.1.1/03; [redacted]; 2015; M-534551-01-1
Title:	Ethephon technical: Acute oral toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.:	S15-00347
Document No.:	M-534551-01-1
Guideline(s):	OECD Guideline No. 213 (1998), OEPP/EPPO 170 (4) (2010), and the review article of VAN DER STEEN (2001)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To determine the acute oral toxicity of ethephon to the bumble bee *Bombus terrestris* L.

Material and Methods:

The test item was ethephon technical concentrate (73.6 % w/w a.s.) of batch no. HR4C21X02. Young adult worker bumble bees *Bombus terrestris* L. were used as test organisms. The test was carried out as a limit test with one nominal dose of 250 µg a.s./bee of the test item, one control (50% w/v sucrose solution) and with one dose of 1.5 µg dimethoate/bee ('Perfekthion') as a reference item. The test item treatment group contained 50 bees. Control and reference treatment groups consisted of 30 bees each. Deionised water was used as solvent for the test and reference item. For dose verification the amount of application solution consumed was determined by weighing the feeders before and after feeding. Mortality and behavioural abnormalities were assessed 24 and 48 hours after dosing. The bees were kept in constant darkness except during the application and the assessments which were conducted in daylight. The temperature was 24.6 to 25.8°C, the relative humidity was 55.7 to 60.9 %.

Results:

Mortality over the whole test duration was 0% in the control and test item group. No behavioural effects were observed.

Ethephon: Mortality and actual uptake in an oral toxicity test on bumble bee

Treatment group	Doses [µg a.s./bee]		Mortality [%]	
	Nominal dose	Actual uptake	24 h	48 h
Control	-	-	0.0	0.0
Test item	250	167	0.0	0.0
Reference item	1.5	1.3	56.7	56.7

[a] Assessed through reweighing of the feeders

Validity criteria:

Mortality of the bumble bees in the control:	0.0 % (required: ≤ 10%)
Mortality of the bumble bees in the reference item (48h)	56.7 % (required: ≥ 50%)

Test is valid as control mortality was ≤ 10% and mortality for reference item (dimethoate) was ≥ 50%.

Conclusions:

The oral LD_{50} (48 h) for bumble bee was >167 µg a.s./bee. The oral NOED (48 h) was 167 µg a.s./bee.

CA 8.3.1.1.2 Acute contact toxicity

For information on the study already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The contact toxicity endpoint from this study is included in Table 8.3.1- 1 in grey text. A summary of a new study including a contact toxicity test is summarised in the previous section (CA 8.3.1.1.1). In addition, a summary of a new study on bumble bee is presented below. Endpoints from contact toxicity studies are listed in Table 8.3.1- 1.

Report:	KCA 8.3.1.1.2/02; [redacted]; 2015; M-525423-01-1
Title:	Ethephon technical: Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.:	S14-00624
Document No.:	M-525423-01-1
Guideline(s):	No specific guidelines available. Based on EPPO 170 (4) (2010), OECD Guideline No. 214 (1998) and the review article of [redacted] (2001)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

To determine the toxicity of ethephon on the bumble bee, *Bombus terrestris* L. by contact exposure.

Material and Methods:

The test item was ethephon technical concentrate (73.6 % w/w a.s.) of batch no. HR4C21X02. In the laboratory, the bees were exposed to 100 µg a.s./bee by topical application. Mortality and sub-lethal effects were assessed 24 and 48 hours after application. The control group was exposed to tap water for the same period of time under identical conditions. The test item treatment group contained 50 test organisms, divided in 5 parallel replicates, each containing 10 test organisms. The control group contained 30 test organisms, divided in 3 parallel replicates, each containing 10 test organisms.

Results:

Mortality over the whole test duration was 0% in the control and test item group. No behavioural effects were observed.

Ethephon: LD_{50} values in a contact toxicity test on bumble bee (*Bombus terrestris*)

Exposure route	contact	
Time	24 h	48 h
Applied dose: µg a.s./bee	100.0	100.0
LD_{50} µg a.s./ bee	> 100.0	> 100.0

The test was valid as control mortality was < 10% and mean mortality in the reference test was ≥ 50%.

Conclusions:

The contact LD_{50} (48 h) for bumble bee was > 100.0 µg a.s./bee.

CA 8.3.1.2 Chronic toxicity to bees

No studies on chronic toxicity to bees were evaluated in the previous EU review. A new study is summarised below and in CP 10.3.1.2. The endpoints are included in Table 8.3.1- 1. In this study the active substance was assessed by testing the representative formulation Ethephon SL 480.

Report:	KCA 8.3.1.2/01; [redacted]; 2015; M-534554-01-1
Title:	Ethephon SL 480A G - Assessment of effects on the honeybee Apis mellifera L., in a 10 days chronic feeding test under laboratory conditions
Report No.:	S14-00179
Document No.:	M-534554-01-1
Guideline(s):	No specific guideline available. Based on OECD Guideline No. 213 (1998), CEB No. 230 (2013) and OECD Guideline Proposal (2013)
Guideline deviation(s):	none
GLP/GEP:	**yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.3.1.2. Due to technical reasons the study cannot be removed from the CA dossier. For convencience the summary is still provided below.

Objective:

To determine the effect of Ethephon SL 480 on the honey bee in a 10-day chronic feeding test.

Material and Methods:

The test item was Ethephon SL 480 (492.3 g a.s./L; 41.0 % w/w a.s.) of batch no. B3090017. During 10 days, bees were exposed to 50 % w/v sucrose solution with nominal concentrations of 187.5, 375, 750, 1500 and 3000 mg a.s./kg by continuous and *ad libitum* feeding. The control was exposed to untreated sucrose solution. Mortality and sub-lethal effects were assessed daily. The consumption of sucrose solution, the mean intake of test item and the accumulated mean intake of test item were determined. Solutions were prepared freshly every day throughout the 10-day period. Samples were taken daily for analysis for ethephon. This analysis was performed around *one year* after the in-life phase and no stability data are available. Hence, the analytical results are considered to be supporting information only. [In-life: 27 May to 24 June 2014; chemical analysis: 22 April to 12 May 2015]

Results:

No control mortality was observed. The cumulative mortality at 187.5, 375, 750, 1500 and 3000 mg a.s./kg solution was 0.0, 0.0, 2.5, 0.0 and 5.0 %, respectively at the final assessment. In the reference item group, mortality was 87.5 %. The study was considered valid because the mean mortality in the

control was ≤15% and the mortality for the reference item was ≥50 %. In the control and at all test item treatment levels no sub-lethal effects were observed. Overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) was at the highest concentration of 3000 mg a.s./kg statistically significantly lower than to the untreated control. Results are in the following table.

Results of a chronic feeding study on adult honeybees:

Treatment mg a.s./kg feeding solution	10-day cumulative mortality %	Overall mean consumption of feeding solution mg/bee/day	Dietary dose (DD) µg a.s./bee/day	Accumulated mean uptake µg a.s./bee
C[1] (0.0)	0.0	40.9	-	-
R[2] (0.8)	87.5	35.4	0.029	0.29
Ethephon SL 480[3]				
187.5	0.0	39.7	7.44	74.39
375	0.0	42.3	15.85	158.52
750	2.5	42.5	31.90	319.02
1500	0.0	38.5	57.70	577.03
3000	5.0	31.8*	95.53	955.29
LC_{50}	> 3000 mg a.s./kg feeding solution			
LDD_{50}	> 95.53 µg a.s./bee/day			
NOEC	3000 mg a.s./kg feeding solution			
NOEDD	95.53 µg a.s./bee/day			

[1] Feeding solution: 50 % w/v aqueous sucrose solution
[2] Feeding solution: 50 % w/v aqueous sucrose solution containing Perfekthion (a.s. dimethoate)
[3] Feeding solution: 50 % w/v aqueous sucrose solution containing Ethephon SL 480
* 22% lower than the control, which was statistically significant (Williams t-test α = 0.05)
LDD_{50} = Median Lethal Dietary Dose

Analytical Results: The analysed concentration of ethephon for 10 consecutive days per individual test item treatment level was within the range of 74 – 85 % of the nominal concentration. No residues of ethephon above the LOQ (10 µg/kg) were found in any of the control samples.

Conclusions:

The LC_{50} for 10 days of continuous exposure was >3000 mg a.s./kg feeding solution. The corresponding LDD_{50}, based on the actual consumption, was >95.53 µg a.s./bee/day The NOEC for mortality after 10 days was 3000 mg a.s./kg feeding solution. The corresponding NOEDD, based on the actual consumption, was 95.53 µg a.s./bee/day. Consumption of sucrose solution containing 3000 mg a.s./kg was 22% lower than that consumption of untreated sucrose solution in the control.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

No studies on honeybee development and other honeybee life stages were evaluated in the previous EU review. A honey bee brood feeding study ([redacted], 2015) was conducted in 2013, but was later judged to be unreliable. To replace this study an acute toxicity study on honey bee larvae ([redacted], 2015) and a honey bee tunnel test ([redacted], 2015) were subsequently conducted. The brood feeding study and acute larval toxicity study are summarised below. A summary of the tunnel test is given in MCP 10.3.1.5.

Report:	KCA 8.3.1.3/01; [redacted]; 2015; M-528291-01-1
Title:	Ethephon SL 480B G - A honey bee brood feeding study to evaluate potential effects on brood development and mortality of the honey bee, Apis mellifera L. (Hymenoptera: Apidae)
Report No.:	20130045
Document No.:	M-528291-01-1
Guideline(s):	Based on the method according to [redacted] et al. (1992)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.3.1.3. Due to technical reasons the study cannot be removed from the CA dossier. For convencience the summary is still provided below.

After study finalisation, it was realised that the sucrose solution containing 2.4 g a.s./L should have been pH-buffered. The pH of a 2.4 g a.s./L aqueous solution of Ethephon SL 480 is 2.0 ([redacted], 2015, M-542286-01-1, KCA 8.3.1.3/03, KCP 10.3.1.3/02). Uptake of 1 L of the treated sucrose solution by each colony was clearly slower than uptake of untreated sucrose solution by control colonies. This was probably related to acidity. The possibility of consequent experimental artefacts could not be excluded. Hence, the study was concluded as unreliable. Subsequently, to replace the study, an acute larval toxicity study ([redacted], 2015) and a honeybee tunnel test ([redacted], 2015) were done.

In the honey bee tunnel test ([redacted], 2015), Ethephon SL 480 was sprayed onto flowering *Phacelia* at 120 or 480 g a.s./ha in the presence of one colony per tunnel. The nectar from foraging bees was analysed for ethephon. The highest measured concentration in nectar was 3 mg a.s./kg (day 0). This realistic worst-case level of ethephon in nectar is 800x lower than the concentration in the sugar solution used in the brood feeding study (2400 mg a.s./L). Hence, *in hindsight*, the exposure concentration in the brood feeding study can be regarded as completely unrealistic.

Objective:

To investigate the effect of Ethephon SL 480 on honey bee brood when exposed by via the diet.

Material and Methods:

The test item was Ethephon SL 480 (487.7 g a.s./L, analysed) from batch no. NK49CX0211. The test item (4.93 mL) was mixed with each 1 L of 50% (w/v) sucrose solution to give a concentration of 2.4 g a.s./L. One litre of this solution was then fed to each of three colonies per test group. Mortality of adult bees, pupae and larvae was assessed 21 days after introduction of the test item. Also bee brood development (eggs, young and old larvae) was recorded one day before introduction of the test item, and 4, 8, 15 and 21 days after introduction of the test item. Three control colonies were given untreated sucrose solution. 3.0 g of Insegar (25% fenoxycarb) in 1 L of sucrose solution was used as a reference substance (i.e. 0.75 g fenoxycarb/L). The bees were free flying, with access to natural foraging recourses (e.g. nature and pollen) in the surroundings. Due to the time of the year, mass-flowering crops was already fading (Dates of experimental work: June 17 to July 12, 2013).

Results:

Results are summarised in the table on the following page.

Ethephon SL 480: Results of a brood feeding study on honey bee (*Apis mellifera*):

Assessment period	Control n=3	Test Item n=3	Reference Item n=3
	Worker Mortality / Colony (Means ± SD)		
Pre-Application (DAT -3 to 0)	22.33 ± 4.68	13.00 ± 2.65	17.67 ± 8.08
Post-Application (DAT 1 to 22)	13.52 ± 4.43	10.29 ± 3.39	18.18 ± 3.09
	Pupal Mortality / Colony (Means ± SD)		
Pre-Application (DAT -3 to 0ba)	0.42 ± 0.38	1.08 ± 0.52	0.25 ± 0.25
Post-Application (DAT 1 to 22)	0.38 ± 0.17	0.53 ± 0.38	53.58 ± 20.07$^{\Delta}$
	Development of selected Eggs (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	11.67 ± 2.52	11.33 ± 15.95$^{\Delta}$	34.67 ± 23.71$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.42 ± 0.13	3.43 ± 0.80	3.27 ± 1.19
Compensation Index at BFD 22 (DAT 21)	4.57 ± 0.09	3.84 ± 0.58	3.36 ± 1.25
	Development of selected Young Larvae (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	3.33 ± 1.5	9.33 ± 9.24$^{\Delta}$	12.00 ± 6.08$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.83 ± 0.08	4.60 ± 0.35	4.40 ± 0.30*
Compensation Index at BFD 22 (DAT 21)	4.85 ± 0.09	4.61 ± 0.36	4.42 ± 0.29*
	Development of selected Old Larvae (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	1.67 ± 2.08	5.67 ± 4.73$^{\Delta}$	14.67 ± 11.59$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.92 ± 0.10	4.72 ± 0.24	4.26 ± 0.58*
Compensation Index at BFD 22 (DAT 21)	4.94 ± 0.07	4.81 ± 0.13	4.30 ± 0.61*

$^{\Delta}$ Statistically significantly greater as compared to the control
* Statistically significantly smaller as compared to the control
DAT Days After Treatment
BFD Brood area Fixing Day

Uptake of sucrose solutions: The results for uptake of the 1 L of sucrose solutions per colony are presented below:

Results for consumption of 1 L of 50 % sucrose solution

Treatment	Replicate	Test solution consumed (Y/N)	Test solution consumed within (h)	Leftover volume (mL)*	No. of dead bees in feeder
Control	1	Y	48	0	0
	2	Y	24	0	0
	3	Y	24	0	0
Test item	1	Y	48	0	1
	2	Y	72	0	0
	3	Y	72	0	4
Reference item	1	Y	48	0	57
	2	Y	48	0	53
	3	Y	48	0	61

* measured on DAT 22; the initial volume of feeding solution per colony was 1000 mL per colony

Two of the colonies presented with sucrose solution containing the test item took 72 hours to take up the complete 1 L volume. This contrasts with the control, for which two colonies took 24 hours to take up the same volume.

Bee behaviour: In all treatments, no abnormal behaviour was observed during the whole study period, except slightly increased aggressiveness in two of the reference item replicates between DAT 10-12.

Colony strength: During the course of the study, the mean colony strength in the control, test item and reference item treatment displayed a relative increase of 30%, 19% and 17%, respectively, at study termination (DAT 22). No statistically significant differences were detected between the treatments.

Brood nest (eggs/larvae/pupae): During the course of the study, the estimated mean comb area comprising brood per colony displayed a relative change of + 16%, - 2% and - 30%, respectively, at study termination (DAT 22). There was a statistically significant negative effect on the relative change of the brood nest size of the reference item treatment as compared to the control.

Stores (pollen/nectar/honey): During the course of the study, the estimated mean comb area comprising food per colony displayed a relative increase of 51%, 63% and 65%, respectively, at study termination (DAT 22). For this parameter, no statistically significant differences were detected between the test item treatment or the reference item treatment, compared with the control. In this

study, the major influence of the reference item could be seen as a high level of pupal mortality which is a known effect for this substance.

Vacant cells: During the course of the study, the estimated mean comb area comprising of vacant cells per colony displayed a relative change of - 43%, - 24% and + 17%, for the control, test item and reference item treatment, respectively, at study termination (DAT 22). There was a statistically significant negative effect on the relative change of vacant cells of the reference item treatment as compared to the control.

Brood Termination Rate (BTR): As compared to the control, in the test item treatment a statistically significant increase of BTR was detected for initially selected eggs (from BFD 5 onwards), young larvae (from BFD 9 onwards) and old larvae (from BFD 5 onwards). Although BTR was statistically significantly higher than observed in the control for both young and old larvae in the test item treatment the actual levels were quite low (9.33 and 5.67%, respectively) which may not be biologically significant for the development of the colony. As compared to the control, in the reference item treatment a statistically significant increase of BTR was detected for initially selected eggs (from BFD 16 onwards), young larvae (from BFD 9 onwards), and old larvae (from BFD 9 onwards). Although this supports that the test system was sensitive to detect potential effects of plant protection products on honey bee brood the overall levels of effects on BTR seen in the reference item treatment were relatively low. In this study, the primary indicator of effect was of that on pupal mortality, which was not observed in either the control or test item treatment.

Bee brood index: While the Brood Indices of initially selected young and old larvae in the test item treatment displayed increases comparable to the control, thus indicating a successful development of the brood, the Brood Index of eggs remained lower as compared to the control. Statistical analyses showed that Brood Indices in the test item treatment were not significantly decreased as compared to the control, except for a single assessment at BFD 9, where a statistically significant decrease was detected for eggs. Compared to the control, mean Brood Indices of the reference item treatment were not statistically significantly decreased for selected eggs, but were significantly decreased for young larvae at BFD 22 and for old larvae from BFD 9 onwards.

Brood Compensation Index: Overall, except for selected eggs, the Brood Compensation Indices of the control and test item displayed comparable increases, indicating a successful compensation of previous brood losses. Statistical analyses showed that Brood Compensation Indices in the test item treatment were not significantly decreased after completing a whole brood cycle (i.e. at BFD 22) as compared to the control (although a transient difference was observed between control and test item treatment at BFD 9). In contrast, the mean Brood Compensation Indices of the reference item treatment exhibited a statistically significant decrease as compared to the control for young larvae at BFD 22 and for old larvae from BFD 9 onwards, but not for eggs.

Conclusions:

Overall, according to the results of this study, it seems unlikely that Ethephon SL 480 fed under worst case test conditions at a concentration of 2.4 g a.s./L (2400 mg a.s./L) will cause irreversible adverse effects on honey bee colony vitality or survival.

Evaluator comment:

The BTR for marked eggs was higher in the ethephon-treated colonies than the control. But also, consumption of sucrose solution was also markedly slower in these colonies than in the control. It cannot be excluded that the acidity (pH 2.0) of the ethephon-treated solution had an influence on the uptake rate of the treated solutions. Also, this low pH is likely to have resulted in general 'irritation' of adults and brood in the dosed colonies. These factors had the potential to increase the BTR. As such, the higher BTR in the test item colonies than the control colonies can be regarded as an artefact of the 'physico-chemical' impact of low pH. For this reason, the study was judged to be unreliable. In addition, the study is lacking in *relevance* as the tested concentration in sucrose was 800x higher than *measured realistic* worst-case levels in nectar from foraging bees in the subsequent tunnel test ([redacted], 2015). Overall, the brood feeding study summarised above is not considered suitable for use in the risk assessment.

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Report: KCA 8.3.1.3/02; [redacted]; 2015; M-540682-01-1
Title: Ethephon technical concentrate - Honey bee (Apis mellifera L.) larval toxicity test (single exposure)
Report No.: S15-02462
Document No: M-540682-01-1
Guideline(s): OECD Guideline No. 237 (2013)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

To determine the effects of ethephon on the larvae of honey bee, *Apis mellifera* L., from a single feeding exposure in a 7-day *in vitro* limit test.

Material and Methods:

The test item was ethephon technical concentrate (analysed: 73.6 % w/w a.s.) of batch no. HR4C21X02. The test organisms were first instar larvae. There was one control group, one test item group with 100 µg a.s./larva, and one reference item group with 8.8 µg dimethoate/larva. This limit test had a duration of 7 days from grafting on Day 1 to the final assessment on Day 7. On Day 4, one single dose of test item in larval diet was applied to larvae of the test item group. Samples of this treated diet were analysed for ethephon by LC-MS/MS. One single dose of the reference item in

larval diet was applied to the larvae of the reference item group. A control group was dosed with untreated larval diet. Each group consisted of 48 larvae from three different colonies (16 larvae from a single colony per replicate, with 3 replicates). Assessment of mortality was done on Day 5, Day 6 and Day 7 (i.e. 24 h, 48 h and 72 h after application of treated diet). The presence of uneaten food was qualitatively recorded at the end of the test on Day 7.

Fisher's Exact Test (one-sided, $\alpha = 0.05$) was used to evaluate whether there was a significant difference between the mortality in the test item group and the control. This test was also used to determine whether there was a significant difference between the mortality in the reference item group and the control.

Results:

The measured concentration of ethephon in the applied diet was 95% of the nominal concentration. Hence, biological results are expressed as the nominal concentration and intended dose of test item.

No mortality occurred in the control group (i.e. mortality was less than validity criterion of 15%). In comparison, the test item group did not show a statistically significantly increased mortality. In the reference item group, the mortality was 72.9% (i.e. greater than validity criterion of 50%). On day 7 (D7) uneaten food was observed in the reference item group. During the mortality assessments, no noticeable observations such as deviating larval size and appearance were made. A summary of the results is presented in the following table.

Effects of ethephon on honey bee larvae, *Apis mellifera* L., after a single exposure

Treatment group	Dose		Cumulative mortality [%]		
			Day 5	Day 6	Day 7
Control	---	---	0.0	0.0	0.0
Test item (ethephon)	100.0	[µg a.s./larva] [a]	0.0	0.0	0.0
Reference item (dimethoate)	8.8	[µg/larva] [a]	18.8*	62.5*	72.9*
Endpoints for ethephon for Day 7					
NOED	100 µg a.s./larva				
NOEC	3030.3 mg a.s./kg diet				
LD_{50}	> 100 µg a.s./larva				
LC_{50}	> 3030.3 a.s./kg diet				

* Significantly increased compared to control (Fisher's Exact Test, one-sided greater, $\alpha = 0.05$)

[a] Taking account of the analysed active substance content of the test item (i.e. 73.6% w/w a.s.)

Conclusions:

The LD_{50} for honey bee larvae was >100 µg a.s./larva. The LC_{50} was >3030.3 mg a.s./kg diet.
The NOED was 100 µg a.s./larva. The NOEC was 3030.3 mg a.s./kg diet.

The following is the summary of a report of a pH measurement for a 0.5 % v/v dilution of ethephon SL480 in water. This dilution (equivalent to 2.4 g a.s./L) is the same concentration as the ethephon SL 480 dosing solution used in the honey bee brood feeding study (KCA 8.3.1.3/01). The measurement has been made to enable complete interpretation of KCA 8.3.1.3/01.

Report:	KCA 8.3.1.3/03; [redacted]; 2015; M-542286-01-1
Title:	pH-value of ethephon SL 480 (480 g/L) - Final report
Report No.:	FOR0915(PCR00)N01
Document No.:	M-542286-01-1
Guideline(s):	CIPAC-Handbook Volume J / 2000 MT 75.3
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective:
To determine the pH of a 0.5% v/v dilution of ethephon SL 480 in water (in terms of active substance, the concentration in water was 2.4 g a.s/L).

Materials and methods:
The test item was ethephon SL 480 (batch no. EM4H003116; analysed a.s. content: 39.4% w/w). The determination of the pH-value was carried out electrometrically by means of a single-rod measuring chain. In this method a glass electrode was immersed into a 0.5% v/v dilution of the test item in deionised water. The sample was mixed by means of a magnetic stirrer for one minute. The stirrer was switched off and after a further minute the pH-value was measured and recorded directly on the pH-meter. The final pH value resulting from three valid measurements was reported.

Results:
The measured pH was 2.0.

Conclusion:
The measured pH for a 0.5% v/v dilution of ethephon 480 SL in deionised water was 2.0. This dilution in terms of active substance was 2.4 g a.s./L.

CA 8.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess "sub-lethal effects" in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported. A tunnel test on honey bees is presented in Document MCP, and the results will be included in the risk assessment.

CA 8.3.2 Effects on non-target arthropods other than bees

For information on studies already evaluated during the previous EU review, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

Point 4 on p54 of the active substance data requirements under Regulation 1107/2009, specifies that the test item in non-target arthropod studies can be the representative plant protection product. Hence, data presented in the current EU review are from studies using Ethephon SL 480. The data requirements for the active substance are for laboratory glass plate studies on *Aphidius rhopalosiphi* and *Typhlodromus pyri*. A single-rate glass plate study for each of these species was evaluated during the previous EU review. In the study on *A. rhopalosiphi* >50% mortality occurred at 726 g a.s./ha. Hence, for the current EU review, a new laboratory study on this species has been conducted with a *range* of application rates (including lower rates) in order to derive an LR_{50} (Waibel, 2015).

The endpoints from the available glass plate studies on *A. rhopalosiphi* and *T. pyri* are listed in Table 8.3.2- 1. Endpoints from the two studies evaluated during the previous EU review are stated in grey text. Endpoints from the new study are stated in black text. A summary of the new study is provided later in this section. Extended laboratory (realistic substrate) studies on *Aphidius rhopalosiphi* and studies on other non-target arthropod species are summarised in Section MCP 10.3.2.

Table 8.3.2- 1: Ethephon (Ethephon SL 480): Endpoints from laboratory studies on non-target arthropods

Test species	Study type, application rate	Endpoint	Reference
Aphidius rhopalosiphi	Laboratory, glass plate. 726 g a.s./ha	47.2% (mortality, 5.4% decrease in parasitisation efficiency	LoEP KCA 8.3.2.1/01 M-172516-01-1
Aphidius rhopalosiphi	Laboratory, glass plate 5 rates: 48 to 480 g a.s./ha	Rate g a.s./ha: 48 85 152 270 480 Corr. Mort. %: 0 5.0 1.7 5.0 55.0 LR_{50} : 465 g a.s./ha	[redacted] (2015) KCA 8.3.2.1/02 KCP 10.3.2.1/05 M-528489-01-1
Typhlodromus pyri	Laboratory, glass plate 726 g a.s./ha	13.7 % mortality; No significant adverse effects on reproduction (R=0.67)	LoEP KCP 8.3.2.2/01 M-172467-01-1

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

A new rate-response laboratory glass plate study on *A. rhopalosiphi* is summarised below.

Report:	KCA 8.3.2.1/03; [redacted]; 2015; M-528489-01-1
Title:	Toxicity to the parasitoid wasp Aphidius rhopalosiphi (Hymenoptera: Braconidae) using a laboratory test ethephon SL 480 g/L
Report No.:	CW15/020
Document No.:	M-528489-01-1
Guideline(s):	IOBC draft ([redacted] et al. 2000); [redacted] et al. (2000)
Guideline deviation(s):	none
GLP/GEP:	**yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.3.2.1. Due to technical reasons the study cannot be removed from the CA dossier. For convenience the summary is still provided below.

Objective:

To investigate the toxicity of Ethephon SL 480 to *A. rhopalosiphi* when exposed to treated glass plates.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. The test item was applied to glass plates at rates of 48, 85, 152, 270 and 480 g a.s./ha, and allowed to dry. The effects on *A. rhopalosiphi* (<48 h old) of contact exposure to these plates was compared to those of a water-treated control. A toxic reference (dimethoate) applied at 0.05 g a.s./ha was also included. There were 4 replicates of 15 wasps, for the treatment group, and for the control. Mortality was assessed 2, 24 and 48 h after the start of exposure. Temperature was 19.5-20.5 °C and relative humidity was 71-83%. The light/dark cycle was 16:8 h with light intensity of 1026-1495 Lux.

Results:

Ethephon SL 480: Results of a laboratory glass-plate rate-response study on *Aphidius rhopalosiphi*

Exposure	Dried spray deposits on glass plates		
Treatment [g a.s./ha]	**Mortality after 48 hours [%]**	**Corrected mortality [%]**	**P-Value[1]**
Control	0.0	-	-
48	0.0	0.0	1.000 ns
85	5.0	5.0	0.487 ns
152	1.7	1.7	1.000 ns
270	5.0	5.0	0.487 ns
480	55.0	55.0	0.001*
Toxic reference 0.05 g dimethoate/ha	91.7	91.7	-
LR_{50} = 465 g a.s./ha (95% Confidence Interval: 393 – 611 calculated with Probit analysis)			

[1] Fisher´s Exact test (one-sided, α = 0.05); * = statistically significant; ns = not statistically significant

Conclusions:

The LR_{50} for *A. rhopalosiphi* was calculated to be 465 g a.s./ha.

CA 8.3.2.2 Effects on Typhlodromus pyri

Data on *T. pyri* were provided and evaluated in the previous EU review (endpoint in Table 8.3.2-1).

CA 8.4 Effects on non-target soil meso and macrofauna

CA 8.4.1 Earthworm, sub-lethal effects

For information on studies already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

Two new earthworm reproduction studies have been for conducted for the current EU review. Firstly, a study has been performed using the formulation Ethephon SL 480. The formulation was employed as the test item, as a means of testing the active substance. The rationale for conducting this study was to confirm the result of the study evaluated during the previous EU review. Secondly, a study has been conducted on the soil metabolite HEPA. This study was performed because HEPA is considered to be a 'major' metabolite in soil in Section CA 7 (Environmental Fate and Behaviour).

The endpoints from toxicity studies on earthworms are presented in Table 8.4.1- 1. Endpoints from studies evaluated during the previous EU review are stated in grey text. Endpoints from new studies are stated in black text. Summaries of the two new studies are provided later in this section.

Table 8.4.1- 1: Ethephon and HEPA: Endpoints from earthworm toxicity studies

Test item	Test species, test design	Endpoint		Reference
Ethephon	*Eisenia fetida* acute, 14 d, mixed*	**LD50**	**>165.4 mg a.s./ha equivalent to >60 kg a.s./ha**	LoEP KCA 8.4.1/01 M-187830-01-1
Ethephon	*Eisenia fetida* reproduction 56 d, mixed*	**NOEC**	**200 mg a.s./kg dw soil**	LoEP KCA 8.4.1/02 M-200761-01-1
Ethephon SL 480	*Eisenia fetida* reproduction 56 d, mixed*	**NOEC**	**230.4 mg a.s./kg dw soil**	[redacted] (2014) KCA 8.4.1/03 KCP 10.4.1.1/01 M-486043-01-1
HEPA	*Eisenia fetida* reproduction 56 d, mixed*	**NOEC**	**100 mg/kg dw soil**	[redacted] (2015) KCA 8.4.1/04 M-528145-01-1

dw = dry weight; *At the start, the test item was mixed into the soil to achieve a homogeneous distribution.

A summary of a new earthworm reproduction study, using Ethephon SL 480 as the test item, is presented below:

Report: KCA 8.4.1/03; [redacted]; 2014; M-486043-01-1
Title: Ethephon SL 480A G: Effects on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No.: M-486043-01-1
Document No.: M-486043-01-1
Guideline(s): OECD Test Guideline No. 222 (2004)
Guideline deviation(s): none
GLP/GEP: **yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.4.1.1. Due to technical reasons the study cannot be removed from the CA dossier. For convencience the summary is still provided below.

Objective:

The purpose of this study was to investigate the effects of Ethephon SL 480 on the survival (% mortality), body weight, feeding activity and reproduction of the earthworm *Eisenia fetida*.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Ten worms (clitellate adults, age: approximately 10 months) per replicate (eight replicates for the control, four replicates per test item concentration) were exposed to Ethephon SL 480 in artificial soil. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Nominal concentrations were 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dw soil (7.4, 13.1, 23.0, 41.0, 73.0, 129.6, 230.4 and 410 mg a.s./kg dw soil, respectively). Temperature was 18 - 22°C, with a 16 h light (400-800 lux)/8 h dark cycle. After 28 days, the adult

worms were removed, weighed, counted and the remaining treated artificial soil (without the adult worms) was then returned to the respective test containers for further 28 days. At the end of the test period (i.e. after 56 days) the hatched juvenile worms were extracted from the artificial soil by placing the test units in a water bath at 50 - 60 °C and counting all emerging worms.

Results:

Validity criteria:

Mortality of the adult worms in the control:	0 % (required: ≤ 10%)
Number of juveniles per replicate in the control:	148 to 246 (required: ≥ 30)
Coefficient of variation for the number of juveniles in the control:	15.3% (required: ≤ 30%)

All study validity criteria were met.

No statistically significant mortality was observed in any treatment group. The bodyweight changes at 28 days were not statistically significantly different compared to the control up to and including the highest test concentration of 410 mg a.s./kg soil (Williams t-test, α = 0.05, two-sided). The number of juveniles produced was not statistically significantly different to the control up to and including 230.4 mg a.s./kg dw soil. At the highest test concentration of 410 mg a.s./kg dw soil the number of juveniles was statistically significantly lower than the control (Williams t-test, α = 0.05, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

Ethephon SL 480: Effects on Survival (% mortality), Biomass and Reproduction of *Eisenia fetida*

Ethephon SL 480 [mg test item/kg dw soil]	**Control**	**18**	**32**	**56**	**100**	**178**	**316**	**562**	**1000**
ethephon, mg a.s./kg dw soil	**0**	**7.4**	**13.1**	**23.0**	**41.0**	**73.0**	**129.6**	**230.4**	**410**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	0.0
Body weight change (day 28) [%]	30.8	31.8	31.3	29.5	33.4	30.0	35.0	33.9	26.6
Mean No. of juveniles (day 56)	209	183	220	201	202	199	172	203	157*
Reproduction in [%] of control	-	87.6	105.0	95.9	96.6	94.9	82.4	97.2	74.8*
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg a.s./kg dw soil]									
NOEC day 28 mortality, weight	**410**								
NOEC day 56 reproduction	**230.4**								

Rounded values were calculated from the exact raw data. * = significantly different to the control (α = 0.05)

The EC_{50} (repro) for Carbendazim 500 FC tested as a toxic reference item was 1.32 mg test item/kg soil dw. The effects of carbendazim confirm the suitable sensitivity of the test system.

Conclusions:

In an earthworm reproduction study with Ethephon SL 480 the overall NOEC for mortality, growth, reproduction and feeding activity was 230.4 mg a.s./kg dw soil.

The RMS requested to report the corresponding EC_{10} and EC_{20} values for this study. As stated in the study report, the EC_{10} was determined to be 273.7 mg product/kg soil (corresponding to 112.2 mg a.s./kg soil) and the EC_{20} was determined to be 1151.5 mg product/kg soil (corresponding to 472.1 mg a.s./kg soil). Confidence intervals could not be determined.

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Report:	KCA 8.4.1/04; [redacted]; 2015; M-528145-01-1
Title:	Ethephon-2-hepa (BCS-BA97658) Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No.:	15 10 48 126 S
Document No.:	M-528145-01-1
Guideline(s):	OECD Test Guideline No. 222 (2004)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of this study was to investigate the effects of HEPA on the survival (% mortality), body weight, feeding activity and reproduction of the earthworm *Eisenia fetida*.

Material and Methods:

The test item was HEPA with Batch code AE F020271 00 1B95 0001 and Origin Batch No. B919 (analysed purity: 95.3 % w/w). In a limit-test, ten worms (clitellate adults, age: approximately 3 months) per replicate (8 replicates for the control and for the treatment group) were exposed to HEPA in artificial soil at a nominal concentration of 100 mg/kg dw soil. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Temperature was 19.1-22°C with a 16 h light (570 lux)/8 h dark cycle. After 28 days, the adult worms were removed, weighed and counted and the remaining treated artificial soil (without the adult worms) was then returned to the respective test containers for a further 28 days. At the end of the test (i.e. total 56 days) the hatched juveniles were extracted from the soil by placing the test units in a water bath at 50 - 60 °C and counting all emerging worms.

Results:

Validity criteria:

Mortality of the adult worms in the control:	0 % (required: ≤ 10%)
Number of juveniles per replicate in the control:	122 to 168 (required: ≥ 30)
Coefficient of variation for the number of juveniles in the control:	10.1 % (required: ≤ 30%)

All study validity criteria were met.

The test item caused no mortality at 100 mg/kg dw soil and there was no mortality in the control. No pathological symptoms and no effects on behaviour (including feeding activity) were observed. The test item caused no statistically significant difference in biomass-change (change in fresh weight after

4 weeks relative to initial fresh weight) compared to the control. There was no statistically significant difference for the number of juveniles compared to the control group.

HEPA: Effects on survival (% mortality), biomass and reproduction of *Eisenia fetida*

HEPA [mg/kg dw soil]	Control	100
Adult mortality (day 28) [%]	0.0	0.0
Body weight change (day 28) [%][2]	23.0	22.5
Mean No. of juveniles per replicate (day 56)	144.5	152.9
Reproduction in [%] of control (day 56)	-	105.8
Endpoints [mg/kg dw soil]		
NOEC (day 28 mortality and weight)	**100**	
NOEC (day 56 reproduction)	**100**	

In the most recent study with a toxic reference (carbendazim, SC 500) the number of juveniles was reduced by 46 and 100 % at 5 and 10 mg product/kg dw soil, respectively, compared to the control. Hence, the test system was suitably sensitive.

Conclusions:

In an earthworm reproduction study with HEPA (limit test) the overall NOEC for mortality, growth, reproduction and feeding activity was 100 mg/kg dw soil.

CA 8.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

No studies on soil meso- and macro-fauna other than earthworms were evaluated during the previous EU review. In the active substance data requirements under Regulation 1107/2009, the need for studies on these organisms is not linked with a DT50 or DT90 trigger in soil. Hence, in order to satisfy these requirements, testing on Collembola (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) has now been performed. In accordance with Point 4 on p54 of the data requirements, the test item in these studies was the representative plant protection product (Ethephon SL 480).

In addition, testing on collembola and soil mites has been performed for the soil metabolite HEPA. These studies were done because HEPA is considered to be a 'major' metabolite in soil in Section CA 7 (Environmental Fate and Behaviour).

Summaries of the four studies are provided at point 8.4.2.1 and endpoints are listed in Table 8.4.2- 1.

Table 8.4.2- 1: Ethephon and HEPA: Endpoints from Collembola and soil mite studies

Test item	Test species, test design	Endpoint	Reference
Collembola, reproduction			
Ethephon SL 480	*Folsomia candida* reproduction, 28 d, mixed*	NOEC 410 mg a.s./kg dw soil	[redacted] (2014) KCA 8.4.2.1/01 KCP 10.4.2.1/01 M-491237-01-1
HEPA	*Folsomia candida* reproduction, 28 d, mixed*	NOEC 100 mg/kg dw soil	[redacted] (2015) KCA 8.4.2.1/03 M-525322-01-1
Soil mites, reproduction			
Ethephon SL 480	*Hypoaspis aculeifer* reproduction, 14 d, mixed*	NOEC 410 mg a.s./kg dw soil	[redacted] (2014) KCA 8.4.2.1/02 KCP 10.4.2.1/02 M-489168-01-1
HEPA	*Hypoaspis aculeifer* reproduction, 14 d, mixed*	NOEC 28.5 mg/kg dw soil	[redacted] (2015) KCA 8.4.2.1/04 M-538939-01-1

*At the start, the test item was mixed into the soil to achieve a homogeneous distribution.

CA 8.4.2.1 Species level testing

Testing on *Folsomia candida* and *Hypoaspis aculeifer* was performed with the representative formulation, Ethephon SL 480, and also with HEPA. Summaries are provided below and endpoints are listed in Table 8.4.2- 1.

Report: KCA 8.4.2.1/01, [redacted], 2014, M-491237-01-1
Title: Ethephon SL 480A (): Effects on reproduction of the Collembola Folsomia candida in artificial soil
Report No.: 90441016
Document No.: M-491237-01-1
Guideline(s): OECD Test Guideline No. 232 (2009)
Guideline deviation(s): none
GLP/GEP: **yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.4.2.1. Due to technical reasons the study cannot be removed from the CA dossier. For convencience the summary is still provided below.

Objective:

The purpose of the study was to determine the effects of Ethephon SL 480 on mortality and reproduction of the Collembola *Folsomia candida* in artificial soil.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Ten collembolans (10-12 days old) per replicate (8 replicates for the control group and 4

replicates for each treatment group) were exposed to control (treated with water) and 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dw. In terms of ethephon, these concentrations were 7.4, 13.1, 23.0, 41.0, 73.0, 129.6, 230.4 and 410 mg a.s./kg dw soil, respectively. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Temperature was 18 to 22°C and lighting was 400–800 lux (16h light: 8h dark). Collembola were fed with approximately 2 mg of dry yeast for each test vessel at the beginning of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 days. An additional test with a toxic reference item was also conducted.

Results:

Validity of the study:

	Required	Achieved
Control Mortality:	≤ 20%	9%
Control Reproduction (Juveniles per Container):	≥ 100	450 to 685
Coefficient of Variation of the Control Reproduction:	≤ 30%	13.8%

All validity criteria were met.

Mortality: Mortality was not statistically significantly increased in any treatment group compared to the control (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

Reproduction: Reproduction was not statistically significantly reduced compared to the control up to and including the highest test concentration of 410 mg a.s./kg dw soil (Williams t-test, $\alpha = 0.05$).

No behavioural abnormalities were observed in any of the treatment groups.

Ethephon SL 480: Effect on Collembola (*Folsomia candida*) in a 28-day reproduction study

Ethephon SL 480 [mg/kg dw soil]	**Control**	**18**	**32**	**56**	**100**	**178**	**316**	**562**	**1000**
ethephon, mg a.s./kg dw soil.	**0**	**7.4**	**13.1**	**23.0**	**41.0**	**73.0**	**129.6**	**230.4**	**410**
Mortality (day 28) [%]	9	15	3	13	3	5	8	5	8
Statistical significance	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
No. of juveniles (day 28)	543	624	612	538	587	612	552	579	557
Reproduction in [%] of control	-	115	113	99	108	113	102	107	102
Statistical significance		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Endpoints [mg a.s./kg dw soil]								
NOEC (mortality)	410								
NOEC (reproduction)	410								

n.s. = not statistically significantly different compared to the control ($\alpha = 0.05$)

Conclusions:

There were no statistically significant differences from the control for survival (% mortality) and reproduction of *Folsomia candida* up to and including 410 mg a.s./kg dw soil (the highest concentration tested). Hence, the NOEC was 410 mg a.s./kg dw soil.

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Report:	KCA 8.4.2.1/02; [redacted]; 2014; M-489168-01-1
Title:	Ethephon SL 480A G: Effects on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil
Report No.:	90441089
Document No.:	M-489168-01-1
Guideline(s):	OECD Test Guideline no. 226 (2008)
Guideline deviation(s):	none
GLP/GEP:	**yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.4.2.1. Due to technical reasons the study cannot be removed from the CA dossier. For convencience the summary is still provided below.

Objective:

The purpose of the study was to determine the effects of Ethephon SL 480 on mortality and reproduction of the predatory mite *Hypoaspis aculeifer*.

Material and Methods:

The test item was Ethephon SL 480 (analysed: 41.0% w/w a.s. or 492.3 g a.s./L) from batch no. B3090017. Ten adult female mites per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments in artificial soil. Concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dw soil were tested. In terms of ethephon, these concentrations were 7.4, 13.1, 23.0, 41.0, 73.0, 129.6, 230.4 and 410 mg a.s./kg dw soil, respectively. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Each test vessel contained 20 g ± 1 g dw artificial soil. The mites were of a uniform age (approx. 9 days after reaching the adult stage). During the test, they were fed with two spatulas of cheese mites (*Tyrophagus putrescentiae*) at the start and 1-2 spatulas on day 2, 5, 7, 8 and 13. Temperature range was 18 to 20°C and the lighting regime was 400–800 Lux with 16 h light:8 h dark. At 14 days, the surviving adults and the living juveniles were extracted by filling the soil into millipore pots with attached plastic containers for collecting the escaping mites. These extraction units were placed in a Kempson extractor. The soil including the mites was exposed to approximately 25°C and 30°C for around 2 days. Extracted *Hypoaspis* were collected in a fixing liquid (glycol and a detergent) and cooled to 16°C. Mites were counted under a binocular microscope.

Results:

Validity of the study: All validity criteria were met.

Validity criteria	Recommended	Obtained
Adult mortality in controls	≤ 20%	4%
Number of juveniles per replicate in controls	≥ 50	184 to 238
Coefficient of variation for no. of juveniles per replicate in controls	≤ 30%	9.0%

Mortality: A statistically significantly higher mortality of 23% was observed at 73 mg a.s./kg dw soil (Fisher's Exact Test, α = 0.05, one-sided greater). This was not considered to be test item related since no statistically significantly higher mortality was observed in the higher treatment levels up to and including 410 mg a.s./kg dw soil.

Reproduction: Reproduction was not statistically significantly different to the control up to and including the highest test level of 410 mg a.s./kg soil (Williams t-test, α = 0.05, one-sided smaller).

Ethephon SL 480: Effect on predatory mite (*Hypoaspis aculeifer*) in a 14-day study

Exposure	Ethephon SL 480, *Hypoaspis aculeifer*		
mg a.s./kg dw soil	% mortality (adults)[1]	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)[2]
Control	4	199 ± 18	-
7.4	5	204 ± 8	103
13.1	10	187 ± 20	94
23.0	8	187 ± 13	94
41.0	5	183 ± 21	92
73.0	23*	182 ± 22	92
129.6	8	180 ± 18	90
230.4	5	185 ± 19	93
410	8	192 ± 10	96
Endpoints [mg a.s./kg dw soil]			
NOEC (mortality)		410	
NOEC (reproduction)		410	

[1] statistical significance tested with Fisher's Exact Test, α = 0.05, one-sided greater

[2] statistical significance tested with Williams t-test, α = 0.05, one-sided smaller

* statistically significantly different compared to the control.

Conclusions:

There were no test item related effects on survival (% mortality) or reproduction of *Hypoaspis aculeifer* up to and including 410 mg a.s./kg dw soil (highest concentration tested). Hence, the NOEC was 410 mg a.s./kg dw soil.

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Report:	KCA 8.4.2.1/03; [redacted]; 2015; M-525322-01-1
Title:	Ethephon-2-hepa (BCS-BA97658): Effects on the reproduction of the collembolan Folsomia candida
Report No.:	15 10 48 124 S
Document No.:	M-525322-01-1
Guideline(s):	OECD Test Guideline No. 232 (2009)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The purpose of the study was to determine the effects of HEPA on survival (% mortality) and reproduction of the Collembola *Folsomia candida* in artificial soil.

Material and Methods:

The test item was HEPA with Batch code AE F020271 00 1B95 0001 and Origin Batch No. B919 (analysed purity: 95.3 % w/w). Ten collembolans (9-12 days old) per replicate (8 replicates for the control group and 8 for the treatment group) were exposed to control (untreated) and 100 mg test item/kg dw artificial soil (limit test). The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Temperature was 19.1 to 22°C, with lighting of 490 lux (16h light: 8h dark). Collembola were fed with approximately 2 mg dry yeast for each test vessel at the start of the test and on day 14. Assessment of adult mortality, behavioural effects and reproduction was performed after 28 days. A test with a toxic reference item (boric acid) was also conducted.

Results:

Validity of the study:

	Required	Achieved
Control Mortality:	≤ 20%	6.3%
Control Reproduction (Juveniles per Container):	≥ 100	1108
Coefficient of Variation of the Control Reproduction:	≤ 30%	15.8%

All validity criteria were met.

Mortality: 6.3 % parental mortality in the control and 6.3 % parental mortality in the test item treatment group was observed. Clearly, there was no statistically significant difference (Chi² 2x2 Test, α = 0.05, one-sided greater). No effects on behaviour were observed.

Reproduction: No statistically significant effects (Student-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg/kg dw soil.

HEPA: Effect on Collembola (*Folsomia candida)* in a 28-day reproduction study

HEPA [mg/kg dw soil]	Control	100
Mortality (day 28) [%]	6.3	6.3
No. of juveniles per replicate (day 28)	1108	1089
Reproduction in [%] of control (day 28)	-	100
Statistical significance [1)]	-	n.s.
	Endpoints [mg/kg dw soil]	
NOEC (reproduction)	100	

n.s. = not statistically significantly different compared to the control. [1)] Student-t-test, α = 0.05, one-sided smaller

Conclusions:

The NOEC in this limit test on HEPA was 100 mg/kg dw soil.

Report: KCA 8.4.2.1/04; [redacted]; 2015; M-538939-01-1
Title: Ethephon-22-hepa (BCS-BA97658): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.: 15 10 48 125 S
Document No.: M-538939-01-1
Guideline(s): OECD Test Guideline no. 226 (2008)
Guideline deviation(s): none
GLP/GEP: yes

Objective:

The purpose was to determine the effects of HEPA on the survival (% mortality) and reproduction of the soil mite *Hypoaspis aculeifer*.

Material and Methods:

The test item was HEPA with Batch code AE F020271 00 1B95 0001 and Origin Batch No. B919 (analysed purity: 95.3% w/w).

1st test run (limit test): Ten adult female mites per replicate (8 control replicates and 8 replicates for the test item concentration) were exposed to control or 100 mg HEPA/kg dw artificial soil. The test item was mixed into the soil before the start of exposure, to achieve a homogenous distribution. Each test vessel contained 20 g ± 1 g dw of soil. The mites were of a uniform age (approx. 9 days after reaching the adult stage). During the test, they were fed with two spatulas of cheese mites (*Tyrophagus putrescentiae*) at the start and 1-2 spatulas on day 2, 5, 7, 8 and 13. Temperature was at 19.7-20.8 °C with 16 h light (528 lux)/8 h dark. At 14 days, the surviving adults and the living juveniles were extracted by filling the soil into millipore pots with attached plastic containers for collecting the

escaping mites. These extraction units were placed in a Kempson extractor. The soil including the mites was exposed to approximately 25°C and 30°C for around 2 days. Extracted *Hypoaspis* were collected in a fixing liquid (glycol and a detergent) and cooled to 16°C. Mites were counted under a binocular microscope.

2nd test run: A 2nd run, with a concentration-response design, was needed due to observed effects in the above limit test. The same method as above was used. There were 4 replicates for each test concentration and 8 replicates for the control. Test concentrations were 1.6, 2.8, 5.1, 9.0, 16.0, 28.5, 50.6 and 90.0 mg HEPA/kg dw soil. Temperature was 19.7 - 21.9 °C with 16 h light (513 lux)/8 h dark.

A test on a toxic reference item (dimethoate) was also conducted.

Results:

Validity of the study: 1st test run

	Recommended	Obtained
Mortality of adult females in the control	≤ 20%	0.0%
Number of juveniles per replicate in the control	≥ 50	273.5
Coefficient of variation for no. of juveniles per replicate in control	≤ 30%	10.1%

Validity of the study: 2nd test run:

	Recommended	Obtained
Mortality of adult females in the control	≤ 20%	1.3%
Number of juveniles per replicate in the control	≥ 50	330.5
Coefficient of variation for no. of juveniles per replicate in control	≤ 30%	12.8%

All validity criteria were met.

HEPA: Results of 1st run of a study on *Hypoaspis aculeifer*:

Endpoint	mg HEPA/kg dw soil	
	Control	100
Mortality of soil mites after 14 days (%)	0.0	1.3
Mean number of juveniles after 14 days	273.5	242.3 *
Coefficient of variation (CV) %	10.1	14.6
Reproduction (% of control)	100	89

*Statistically significantly different to control (Student-t-test, α=0.05). Calculations used unrounded values.

HEPA: Results of 2nd run of a study on *Hypoaspis aculeifer*:

Endpoint	mg HEPA/kg dw soil								
	Control	1.6	2.8	5.1	9.0	16.0	28.5	50.6	90.0
Mortality after 14 days (%)	1.3	7.5	0.0	0.0	2.5	2.5	2.5	0.0	10.0
Mean no. of juveniles after 14 days	330.5	351.3	328.3	327.8	334.8	309.5	314.0	272.8 *	303.3 *
CV (%)	12.8	4.3	6.3	5.1	5.1	8.6	3.9	12.7	4.9
Reproduction (% of control)	100	106	99	99	101	94	95	83	92

* Statistically significantly different to control (Williams-t-test, α=0.05). Calculations used unrounded values

In a separate study, the EC_{50} (repro) of a toxic reference item (dimethoate) was 6.2 mg/kg dw soil, demonstrating the sensitivity of the test system.

Conclusions:

For *Hypoaspis aculeifer*, the NOEC for effects on survival (% mortality) was 100 mg HEPA/kg dw soil. The NOEC and LOEC for reproduction were 28.5 and 50.6 mg HEPA/kg dw soil, respectively. Hence, the overall NOEC was 28.5 mg HEPA/kg dw soil.

The RMS requested to report the corresponding EC_{10} and EC_{20} values for this study. Those endpoints were considered in the statistical evaluation of the original report. However, due to the lack of a dose-response-relation of the data EC_{10} and EC_{20} values could not be determined.

CA 8.5 Effects on soil nitrogen transformation

For information on the study already evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph.

Two additional N-transformation studies are available and are submitted for the current EU review. Endpoints from studies on N-transformation are presented in Table 8.5- 1. The endpoints from the study evaluated during the previous EU review are stated in grey text. Endpoints from the additional studies are stated in black text. Summaries of these two studies are provided later in this section.

Table 8.5- 1: Ethephon and HEPA: Endpoints from studies on nitrogen transformation

Test substance	Test species/study type	Endpoint		References
Ethephon	Study duration 28 d	no unacceptable effects at*:	2.56 mg a.s./kg dw soil 1.92 kg a.s./ha	LoEP KCA 8.5/01 M-179286-01-1
Ethephon SL 480	Study duration 28 d	no unacceptable effects at*:	11.2 mg a.s./kg dw soil 8.42 kg a.s./ha	[redacted] (2008) KCA 8.5/02 KCP 10.5/01 M-302534-01-1

HEPA	Study duration 28 d	no unacceptable effects at*:	2.93 mg/kg dw soil 2.197 kg/ha	[redacted] (2015) KCA 8.5/03 M-526473-01-1

* i.e. differences from the control were <25%.

~~**Report:**~~	~~**KCA 8.5/02; [redacted], T; 2008**~~
~~Title:~~	~~Ethephon SL 480 G: Determination of effects on nitrogen transformation in soil.~~
~~Document No.~~	~~M-302534-01-1~~
~~Guidelines:~~	~~OECD Test Guideline No. 216 (2000)~~
~~GLP~~	~~Yes~~

Report: KCA 8.5/02; [redacted]; 2008; M-302534-01-1
Title: Ethephon SL 480 G: Determination of effects on nitrogen transformation in soil
Report No.: LRT-N-99/08
Document No.: M-302534-01-1
Guideline(s): OECD Test Guideline No. 216 (2000)
Guideline deviation(s): none
GLP/GEP: **yes**

The RMS requested to move the study to the representative formulation dossier under CP 10.5. Due to technical reasons the study cannot be removed from the CA dossier. For convencience the summary is still provided below.

Objective:

To determine the influence of Ethephon SL 480 on nitrogen transformation in an agricultural soil.

Material and Methods:

The test item was Ethephon SL 480 (analysis: 481.2 g a.s./L; Batch No.: 2007-000506). A loamy sand soil was exposed for 28 d to 4.67 µL and 23.33 µL test item/kg dw soil (2.25 and 11.2 mg a.s./kg dw soil, respectively). Application rates were equivalent to 3.5 L and 17.5 L test item/ha (1.68 and 8.42 kg a.s./ha, respectively). Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

Results:

Ethephon SL 480: Effects on non-target soil microorganisms

Test item	Ethephon SL 480	
Test object	Soil Microorganisms; N-Transformation (loamy sand soil)	
Duration	28 days	
µL test item/kg dw soil	4.67	23.33
mg a.s./kg dw soil	2.25	11.2
L test item/ha	3.5	17.5
kg a.s./ha	1.68	8.42
Difference in rates of N formation (%) between control and treatment	2 n.s.	9 *

*statistically significant difference to the control (Welch-t-Test for inhomogeneous variances, α= 0.05)
n.s. : No statistically significant difference to control (Welch-t-Test for inhomogeneous variances, α= 0.05)

Conclusion:

Differences from the control are <25%. Hence, Ethephon SL 480 should not have an impact on N-transformation in soils at 11.2 mg a.s./kg dw soil (8.42 kg a.s./ha).

Report: KCA 8.5/03; [redacted]; 2015; M-526473-01-1
Title: Ethephon-2-hepa (BCS-BA97658): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.: 15 10 48 046 N
Document No.: M-526473-01-1
Guideline(s): OECD Test 216; adopted January 21, 2000
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

To determine the effects of HEPA on soil microflora with regard to nitrogen transformation.

Material and Methods:

The test item was HEPA with Batch code AE F020271 00 1B95 0001 and Origin Batch No. B919 (analysed purity: 95.3 % w/w). A silty sand soil (DIN 4220) was exposed for 28 days to 0.59 and 2.93 mg test item/kg dw soil. Application rates were equivalent to 0.440 kg test item/ha and 2.197 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Results:

The coefficients of variation in the control (NO_3-N) were maximum 3.0 % and thus fulfilled the validity criterion (≤15 %).

At 0.59 mg HEPA/kg dw soil there was a temporary inhibition of the daily nitrate rate at the time interval 7-14 days after application. However, no statistically significant difference in nitrogen transformation was observed at the higher tested rate for this time interval. Also, there were no statistically significant differences for both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of -17.8 % (test concentration 0.59 mg test item/kg soil dry weight) and -14.2 % (test concentration 2.93 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Effects on non-target soil microorganisms treated with HEPA

Test item	HEPA				
Test object	Soil Microorganisms ; Nitrogen-Transformation (loamy sand soil)				
Duration	28 days				
Test concentration	Control	0.59 mg test item/ kg dw soil (eq. to 0.440 kg test item/ha)		2.93 mg test item/ kg dw soil (eq. to 2.197 kg test item/ha)	
	Nitrate-N [1]	Nitrate-N [1]	% difference to control	Nitrate-N [1]	% difference to control
time interval (days)					
0-7	4.61 ± 0.25	5.03 ± 0.38	+9.1 n.s.	4.83 ± 0.14	+4.8 n.s.
7-14	2.28 ± 0.34	1.65 ± 035	-27.4 n.s.	2.06 ± 0.29	-9.6 n.s.
14-28	1.59 ± 0.08	1.31 ± 0.26	-17.8 n.s.	1.36 ± 0.36	-14.2 n.s.

Calculations were performed with unrounded values.

[1] Rate: nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

n.s. No statistically significant difference to the control (Student-t-test for inhomogeneous variances, α= 0.05)

In a separate study, the toxic reference item dinoterb caused a stimulation of nitrogen transformation of +39.1 %, +62.5 % and +112.0 % at 6.80 mg, 16.00 mg and 27.00 mg/kg dw soil, respectively, determined 28 days after application and thus demonstrated the sensitivity of the test system.

Conclusion:

HEPA caused no adverse effects (differences to control were <25 %) on soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The highest test level was 2.93 mg/kg dw soil (2.197 kg test item/ha).

CA 8.6 Effects on terrestrial non-target higher plants

For information on the studies already evaluated during the previous EU review, please refer to the corresponding section in the Baseline Dossier provided by Bayer CropScience, the DAR Addendum of May 2005 and the revised DAR Addendum of January 2006. The studies ([redacted], 1990a and [redacted],

1990b) included seedling emergence and vegetative vigour assays and were conducted with the active substance ethephon formulated as technical concentrate. The lowest endpoints from these assays are stated in Table 8.6- 1 in grey text to denote that they are from previously-evaluated studies.

In 2012, two additional studies with the active substance were conducted on non-target plants ([redacted], 2012a and 2012b). The purpose of these studies was to fulfil a requirement from USEPA to re-run assays for some of the species previously tested with ethephon, technical concentrate in 1990. The studies are summarised in section CA 8.6.2 (KCA 8.6.2/03 and KCA 8.6.2/04). In [redacted], 2012a, seedling emergence assays were run for three species. In [redacted], 2012b, vegetative vigour assays were run for six species. The lowest endpoints from these additional studies are stated in Table 8.6- 1 in black text.

A registered use of ethephon in the EU (e.g. France) is application to immature cucumber plants at 0.24 kg a.s./ha to restrict shoot-extension in order to reduce lodging. The efficacy of such applications was demonstrated in [redacted], 2012b in terms of an ER_{50} of 0.134 kg a.s./ha for shoot length of sprayed cucumber seedlings. In the study, this was by far the lowest endpoint out of all endpoints for the six species tested. Only one other species showed a response for this parameter. This was tomato, for which an ER_{50} of 0.941 kg a.s./ha was derived, indicating a 7x lower sensitivity than cucumber. In terms of context, the ER_{50} for shoot length of cucumber plants relates to an intended use on a target plant. Hence, this is not considered to be relevant to an assessment for non-target plants.

Table 8.6- 1: Summary of non-target plant tests performed with ethephon, technical concentrate

Test organism	Study type	Test duration	Lowest ER_{50} (kg a.s./ha)	Most sensitive species	References
Terrestrial non-target plants; 10 species	seedling emergence; Tier 2 dose response	14 days	ER_{50} = 1.119	Cabbage (shoot length)	[redacted] (1990a) M-187847-01-1 KCA 8.6.2/01 [redacted] (1990b) M-187849-01-1 KCA 8.6.2/02
Terrestrial non-target plants; 3 species	seedling emergence; Tier 2 dose response	21 days	ER_{50} > 2.24	none	[redacted] (2012a) M-443313-02-1 KCA 8.6.2/03
Terrestrial non-target plants; 10 species	vegetative vigour; Tier 2 dose response	14 days	ER_{50} = 1.119	Lettuce (root weight) [a]	LoER [redacted] (1990a) M-187847-01-1 KCA 8.6.2/01 [redacted] (1990b) M-187849-01-1 KCA 8.6.2/02
Terrestrial non-target plants; 6 species	vegetative vigour; Tier 2 dose response	21 days	ER_{50} = 0.134 [b] ER_{50} = 0.496	Cucumber (shoot length) [b] (dry weight)	[redacted] (2012b) M-443312-02-1 KCA 8.6.2/04

[a] For the sake of transparency, the lowest endpoint from this study found for root weight is presented in this table. However, according to current guidelines this is not regarded a relevant endpoint.
[b] The ER_{50} = 0.134 kg a.s./ha relates to an intended use (restriction of shoot extension) on a target plant and is not relevant to non-target plants. Therefore, the lowest ER_{50} for dry weight should be regarded as the relevant endpoint for the active substance ethephon.

CA 8.6.1 Summary of screening data

According to the data requirements for plant protection products (Commission Regulation No 284/2013) screening data are only required for active substances other than those exhibiting herbicidal or plant growth regulator activity. Since ethephon is a plant growth regulator and a complete set of Tier 2 non-target terrestrial plant studies with the representative formulation is available (see MCP), no further data are considered necessary.

CA 8.6.2 Testing on non-target plants

Two studies conducted in 2012 are summarised below. Ethephon ('Base 250') was the test item.

Report:	KCA 8.6.2/03; [redacted]; 2017; M-443313-02-1
Title:	Amendment No. 1 to ethephon 71.3 - Effects on the seedling emergence and growth of three species of non-target terrestrial plants (Tier 2)
Report No.:	SE12/039-A1
Document No.:	M-443313-02-1
Guideline(s):	OPPTS 850.4225, US EPA Ecological Effect Test Guideline, April 1996 Seedling emergence, Tier II and OECD 208 Guidelines for the testing of chemicals, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (July 2006)
Guideline deviation(s):	see section 3
GLP/GEP:	**yes**

Objective:

The objective was to evaluate the effect of ethephon 'Base 250' (71.3 % w/w) [Batch no. 03022F913-SA] on seedling emergence and growth of three non-target plant species following a pre-emergence application of the test item onto the soil surface.

Material and Methods:

The study was conducted from 21 June to 30 July 2012. Three dicotyledonous species were sown in a mixture of 70% sandy-silt loam + 30% sand prior to application of ethephon on the soil surface. Five seeds per pot were sown in 10.5 cm pots in a glasshouse. There were 8 replicate pots per treatment, giving a total of 40 seeds per treatment level. The species were treated with 7 application rates and an untreated control. Dilutions of ethephon were sprayed using a laboratory track sprayer at 200 L/ha.

Application rates and species are shown in the following table:

Species		Application rates g a.s./ha						
		9	23	57	143	358	896	2240
BRSOL	*Brassica oleracea*	X	X	X	X	X	X	X
DAUCS Set 1	*Daucus carota*	X	X	X	X	X	X	X
DAUCS Set 2	*Daucus carota*	X	X	X	X	X	X	X
LACSC Set 1	*Lactuca sativa*	X	X	X	X	X	X	X
LACSC Set 2	*Lactuca sativa*	X	X	X	X	X	X	X

Control pots were sprayed with 200 L/ha of water. Following application, pots were maintained under glasshouse conditions at 23 ± 8ºC during day and 18 ± 8ºC at night with a 16 h photoperiod. Assessments were made 7, 14 and 21 days after application compared with the water-treated controls. The study was terminated 21 days after application. Final assessments were made for emergence, survival, visual phytotoxicity, growth stage, shoot length and shoot dry weight. Statistical analysis was performed to obtain NOER, LOER, LR/ER$_{25}$ and LR/ER$_{50}$ values for emergence, survival, shoot length and shoot dry weight, using ToxRat statistical software.

Results:

All three species met the validity criteria for seedling emergence/survival for US EPA guidelines. The first application for lettuce and carrot (set 1) as well as one repetition for lettuce (set 1) did not meet the validity criterion for emergence in the untreated controls. Due to known germination difficulties with carrot, an additional set of pots (DAUCS set 2) was prepared for this species and for lettuce after the first run was not valid. After application, the pots of set 2 were top-watered until germination reached the validity criteria of emergence in the control pots. Afterwards (DAUCS set 2 and LACSC set 2 after 7 days), the pots of set 2 were bottom-watered.

Analysis of ethephon in applied solution for the highest application rate revealed this to be 102.29% - 103.39% of nominal. Typical symptoms observed on day 21 were chlorosis, leaf deformation and stunting. None, some, or all of these symptoms were exhibited in the species tested. The effects on all species were slight and rarely moderate. The Day 21 NOER, LOER, and ER/LR$_{25}$ and ER/LR$_{50}$ values in g a.s./ha are summarised in the following tables.

Results of a seedling emergence study for ethephon.

BBCH Min-Max on Day 21 at application rates in g a.s./ha								
Species	**control**	**9**	**23**	**57**	**143**	**358**	**896**	**2240**
Brassica oleracea	13[a]-15	13[aa]-15	12[aa]-15	12[a]-15	14-15	13[a]-15	13[aa]-15	10[c]-15
Daucus carota	12-13	11[b]-13	11[b]-12	12-13	11[b]-13	11[b]-13	11[b]-13	11[b]-13
Lactuca sativa	12[c]-16	14-16	12[d]-16	12[d]-16	14-16	12[d]-16	14-16	14-16

[a]Only one Replicate was affected, the majority of the plants were BBCH 14-15
[aa]Only two Replicate were affected, the majority of the plants were BBCH 14-15
[c]Only one Replicate was affected, the majority of the plants were BBCH 13-15
[b]Only one Replicate was affected, the majority of the plants were BBCH 12
[d]Only one Replicate was affected, the majority of the plants were BBCH 14-16

Day 21 phytotoxicity summary (mean % effect)			
g a.s./ha	***Brassica oleracea***	***Daucus carota***	***Lactuca sativa***
Control	**0**	**0**	**0**
9	1.3 e	0	0
23	10.0 de	1.3 d	0
57	6.3 ae	0	0
143	6.3 ae	7.5 de	0
358	10.0 e	8.8 e	5.0 e
896	5.0 e	10.0 e	2.5 e
2240	16.3 de	8.8 ade	1.3 e

a = chlorosis (yellowing of green shoot tissue); *b* = necrosis (brown shoot tissue);
c = bleaching (shoot tissue without pigmentation); *d* = leaf deformation (leaf curl, abnormal leaf shape);
e = stunting (plant height reduced with shorter internode length)
0% = no effect; 10, 20% = slight symptom (s); 30, 40% = moderate symptom (s); 50, 60% = severe symptom (s);
70, 80% = total plant symptom (s); 90% = moribund

Emergence								
Species	**LR_{25} (g a.s./ha)**	**95% CL**		**ER_{50} (g a.s./ha)**	**95% CL**		**LOER (g a.s./ha)**	**NOER (g a.s./ha)**
		lower	**upper**		**lower**	**upper**		
Brassica oleracea	**>2240** [a]	n.d.	n.d.	**>2240** [a]	n.d.	n.d.	**>2240** [a]	**2240** [a]
Daucus carota	**>2240** [a]	n.d.	n.d.	**>2240** [a]	n.d.	n.d.	**>2240** [a]	**2240** [a]
Lactuca sativa	**>2240** [a]	n.d.	n.d.	**>2240** [a]	n.d.	n.d.	**>2240** [a]	**2240** [a]

[a]: calculated values were outside the range tested; n.d. = not determined due to mathematical reasons

Survival								
Species	ER_{25} (g a.s./ha)	95% CL		ER_{50} (g a.s./ha)	95% CL		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Brassica oleracea	>2240 [b]	n.d.	n.d.	>2240 [b]	n.d.	n.d.	>2240 [b]	2240 [b]
Daucus carota	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Lactuca sativa	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]

[a]: calculated values were outside the range tested; [b]: no computations performed due to no mortality
n.d. = not determined due to mathematical reasons

Shoot Length								
Plant Species	ER_{25} (g a.s./ha)	95% CL		ER_{50} (g a.s./ha)	95% CL		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Brassica oleracea	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	2240.0	896.00
Daucus carota	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Lactuca sativa	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]

[a]: calculated values were outside the range tested; n.d. = not determined due to mathematical reasons

Shoot Dry Weight								
Plant Species	ER_{25} (g a.s./ha)	95% CL		ER_{50} (g a.s./ha)	95% CL		LOER (g a.s./ha)	NOER (g a.s./ha)
		lower	upper		lower	upper		
Brassica oleracea	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Daucus carota	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Lactuca sativa	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]

[a]: calculated values were outside the range tested; n.d. = not determined due to mathematical reasons

Conclusion:

Some slight phytotoxic symptoms were observed. The EC_{50} values for emergence, survival, shoot length and shoot dry weight were all >2240 g a.s./ha.

Report:	KCA 8.6.2/04; [redacted]; 2017; M-443312-02-1
Title:	Ethephon 71.3 - Effects on the vegetative vigour of six species of non-target terrestrial plants (Tier 2)
Report No.:	VV12/038
Document No.:	M-443312-02-1
Guideline(s):	OPPTS 850.4250: US EPA Ecological Effects Test Guidelines, April 1996, Vegetative Vigor, Tier II OECD Guideline for the testing of Chemicals, Terrestrial Plant Test OECD 227: Vegetative Vigour Test, July 2006
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective:

The objective was to evaluate the phytotoxicity of ethephon 'Base 250' (71.3 % w/w) [Batch no. 03022F913-SA] on the vegetative vigour of six plant species representing a range of dicotyledonous and monocotyledonous plants, following a post-emergence spray application at the 2-4 leaf stage.

Material and Methods:

The study was conducted from 3 July to 2 August 2012. Six species including four dicotyledonous species and two monocotyledonous species were tested. Plants were grown in a glasshouse in 13 cm pots and were treated at the 2-4 leaf stage. There were 4 plants per pot and 8 replicate pots (32 plants) per treatment. Dilutions of ethephon were sprayed onto the foliage of plants using a laboratory track sprayer at a volume rate of 200 L/ha.

The application rates and test species are shown in the following table:

Species		Application rates g a.s./ha at 200 L/ha volume rate						
		9	23	57	143	358	896	2240
BRSOL	*Brassica oleracea*	X	X	X	X	X	X	X
CUMSA	*Cucumis sativus*	X	X	X	X	X	X	X
LACSC	*Lactuca sativa*	X	X	X	X	X	X	X
LYPES	*Lycopersicon esculentum*	X	X	X	X	X	X	X
LOLPE	*Lolium perenne*	X	X	X	X	X	X	X
ZEAMA	*Zea mays*	X	X	X	X	X	X	X

Control plants were sprayed with 200 L/ha of water. Following application, plants were grown and maintained under glasshouse conditions at 23 ± 8 °C during day and 18 ± 8 °C at night with a 16 h photoperiod. Assessments were made 7, 14 and 21 days after application in comparison with the water-treated controls. The study was terminated 21 days after application. Final assessments were made for survival, visual phytotoxicity, growth stage, shoot length and shoot dry weight. Statistical analysis was performed to obtain NOER, ER/LR_{25} and ER/LR_{50} values for survival, shoot length and shoot dry weight, using ToxRat statistical software.

Results:

Analysis of ethephon in the applied solution for the highest rate tested revealed the concentration to be 103.39% of nominal. This study is valid as the criterion ≥90% survival was achieved in the controls. All species treated with ethephon showed some phytotoxic symptoms. The degree of these symptoms differed with application rate and species. The following tables summarise the day 21 No Observed Effect Rate (NOER), Lowest Observed Effect Rate (LOER), ER/LR_{25} and ER/LR_{50} values for survival, shoot length and shoot dry weight. Endpoints are expressed as g a.s./ha.
Results of a Tier-2 vegetative vigour test on ethephon:

Survival								
Species	LR_{25} g a.s./ha	95% Confidence Limits		LR_{50} g a.s./ha	95% Confidence Limits		LOER g a.s./ha	NOER g as/ha
		lower	upper		lower	upper		
Brassica oleracea	>2240 [b]	n.d.	n.d.	>2240 [b]	n.d.	n.d.	>2240 [b]	2240 [b]
Cucumis sativus	1979.51	1472.83	n.d.	>2240 [a]	n.d.	n.d.	2240.0	896.0
Lactuca sativa	>2240 [b]	n.d.	n.d.	>2240 [b]	n.d.	n.d.	>2240 [b]	2240 [b]
Lycopersicon esculentum	>2240 [b]	n.d.	n.d.	>2240 [b]	n.d.	n.d.	>2240 [b]	2240 [b]
Lolium perenne	>2240 [b]	n.d.	n.d.	>2240 [b]	n.d.	n.d.	>2240 [b]	2240 [b]
Zea mays	>2240 [b]	n.d.	n.d.	>2240 [b]	n.d.	n.d.	>2240 [b]	2240 [b]

[b]: No mortality observed. n.d. : confidence limits not determined due to mathematical reasons or outside the range tested

Shoot Length								
Species	ER_{25} g a.s./ha	95% Confidence Limits		ER_{50} g a.s./ha	95% Confidence Limits		LOER g a.s/ha	NOER g a.s./ha
		lower	upper		lower	upper		
Brassica oleracea	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	2240.0	896.0
Cucumis sativus	21.10	4.17	46.80	134.08	64.89	274.13	<9.0 [a]	<9.0 [a]
Lactuca sativa	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Lycopersicon esculentum	274.51	200.48	349.36	940.88	764.98	1192.06	358.0	143.0
Lolium perenne	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Zea mays	>2240 [a]	1977.92	n.d.	>2240 [a]	n.d.	n.d.	358.0	143.0

[a]: calculated values were outside the range tested; [b]: no computations performed due to no mortality

n.d.: confidence limits not determined due to mathematical reason.

Shoot Dry Weight								
Species	ER_{25} g a.s./ha	95% Confidence Limits		ER_{50} g a.s./ha	95% Confidence Limits		LOER g a.s./ha	NOER g a.s./ ha
		lower	upper		lower	upper		
Brassica oleracea	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	2240.0	896.0
Cucumis sativus	87.51	18.41	183.76	495.87	245.80	1309.96	143.0	57.0
Lactuca sativa	1248.40	n.d.	n.d.	>2240 [a]	n.d.	n.d.	896.00	358.0
Lycopersicon esculentum	552.52	473.48	627.28	1259.03	1135.44	1407.02	358.0	143.0
Lolium perenne	>2240 [a]	n.d.	n.d.	>2240 [a]	n.d.	n.d.	>2240 [a]	2240 [a]
Zea mays	>2240 [a]	1519.31	n.d.	>2240 [a]	n.d.	n.d.	2240.0	896.0

[a]: calculated values were outside the range tested; [b]: no computations performed due to no mortality
n.d.: confidence limits not determined due to mathematical reasons or outside the range tested

Conclusion:

This Tier 2 vegetative vigour study on six non-target terrestrial plant species under glasshouse conditions showed that *Cucumis sativus* (cucumber) was the most sensitive species tested. For this species the EC_{50} was 134.08 g a.s./ha for shoot length and 495.87 g a.s./ha for shoot dry weight.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

No data are available on other terrestrial organisms. Hence, no data are provided for this data-point.

CA 8.8 Effects on biological methods for sewage treatment

For information on the study evaluated during the previous EU review, please refer to corresponding section in the Baseline Dossier provided by Bayer CropScience and the Monograph. The results of this previously-evaluated study are summarised in Table 8.8-1 below.

Table 8.8-1 Ethephon: Results for a test on activated sludge:

Species	Endpoint	Test Guideline	Reference
Activated sludge	3h-EC_{50} > 716 mg a.s./L (respiration inhibition)	OECD 209	KCA 8.8/01 M-172425-01-1

CA 8.9 Monitoring data

Ethephon does not raise concerns for any group of non-target organisms. Consistent with this, there are no monitoring data available which indicate adverse effects (to the best knowledge of the Notifier).

Appendix 1:

Ecotoxicology Position Paper:
Ethephon: Evaluation of Endocrine Activity for Environmental Organisms

Bayer CropScience

ECOTOXICOLOGY

POSITION PAPER

Subject :

Ethephon

Evaluation of Endocrine Activity for Environmental Organisms

Authors:

[redacted]

Bayer CropScience

Date : 2016-01-15

Introduction

Following EU regulation 1107/2009, an assessment has to be provided concerning potential endocrine disrupting properties of the active substance concerned. Therefore such an assessment is presented below for use in the dossier for Annex I renewal (AIR) of Ethephon (ETP).

WHO/IPCS (2002)[2] provided the currently widely accepted definition "*An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations.*" An adverse effect has been defined also by WHO/IPCS (2009)[3]: *"Change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences."*
Both definitions were used as the basis for evaluating the potential impact of Ethephon to wildlife.

Discussion

Wild Mammals

A detailed analysis of all the apical toxicological studies (subchronic, chronic / oncogenicity, reproduction and developmental toxicity) on Ethephon revealed no evidence of any reproducible endocrine effect. Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of Ethephon in mammals.

Birds

The population relevant effects of Ethephon on birds were studied in reproductive toxicity studies on Japanese quail, Bobwhite quail and Mallard ducks. For all three species there were no adverse effects on adult birds, offspring or reproductive parameters up to and including the highest test level of 1000 ppm a.s. As reproduction was not affected in three avian species, it is concluded that there are no population relevant adverse effects of Ethephon. No additional studies are deemed necessary.

Fish

Population relevant effects of Ethephon on fish were studied in an early life-stage test (ELS) with fathead minnow (*Pimephales promelas*) under continuous exposure, resulting in a NOEC of 43 mg/L for mortality and growth (length and weight). At the highest test level (LOEC) of 86 mg/L, 100% mortality of the fish larvae occurred, with the high effect threshold indicating a non-specific mode of action in fish.

[2] WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2002. Global Assessment of the State-of-the-science of Endocrine Disruptors. WHO/PCS/EDC/02.2, 180 pp.

[3] WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. 689 pp.

Based on the absence of relevant effects it can be concluded that Ethephon is not a (potential) endocrine disrupter in fish.

No further testing is indicated to evaluate the endocrine disrupter potential of Ethephon to fish.

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test exist, this test was developed to evaluate to potential effect on the thyroid system, and not to measure population relevant effects. Therefore no further studies can be suggested at this time for these groups of organisms.

Conclusion

Based on the analysis of the complete toxicological data set, there is no evidence of any endocrine disrupting potential of Ethephon in mammals. Likewise in studies with birds, fish and other aquatic organisms no indication of an endocrine activity was found. Therefore it is concluded that Ethephon has no endocrine disrupting activity in environmental organisms.
Further special testing for endocrine disrupting properties is therefore not warranted.