Document Title

Summary of the ecotoxicological studies for fosetyl

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)

[redacted]

Bayer CropScience

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Version history

Date (yyyy-mm-dd)	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2015-10-01	Original Document MCA – Section 9 of Supplementary Dossier	M-534854-01-1
2016-07-20	Dossier update according to "Request for additional information on the supplementary dossier submitted by Bayer CropScience for the approval renewal of the active substance Fosetyl (2015-5865)" by RMS France on 2016-04-04 and its follow up on 2016-06-02: - BCS responses to RMS requests have been added throughout Section 8. - Summaries (including detailed result tables) of the studies used for the first approval of fosetyl and presented in the DAR and addenda to the DAR which are still relevant for the List of Endpoints have been added throughout Section 8. - Endpoints from study [redacted], 1988; M-163531-01-1, KCA 8.2.6.2/01, added to Table 8.2- 1.	M-534854-02-1

[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

Fosetyl was included in Annex I to Directive 91/414/EEC in 2006 (Directive 2006/64/CE of 18 July 2006, Entry into Force on 1 May 2007). This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fosetyl under Directive 91/414/EEC and which were therefore not evaluated during the first EU review. All data which were already submitted by Bayer CropScience (BCS) for the Annex I inclusion under Directive 91/414/EEC are contained in the DAR, its Addenda and are included in the Baseline Dossier provided by BCS. These data are only mentioned in the Supplementary Dossier for the sake of completeness and only general information (e.g. author, reference etc.) is available for these data. In order to facilitate discrimination between new data and data submitted during the Annex I inclusion process under Directive 91/414/EEC, the old data are written in grey typeface. For all new studies, detailed summaries are provided within this Supplementary Dossier. However, for a better understanding of the ecotoxicological behaviour of fosetyl, short summaries including the results of all studies are given at the beginning of the relevant sections. Additional information requested by the RMS France on 2016-04-04 and its follow up on 2016-06-02 during the evaluation of the Supplementary Dossier is highlighted in yellow.

Fosetyl is the ISO common name for ethyl hydrogen phosphonate (IUPAC). Due to the fact that the aluminium salt, a variant of fosetyl, is used in the formulated product, it should be noted that the data in this section belong to the variant fosetyl-aluminium (fosetyl-Al), unless otherwise specified.

In original reports study authors may have used different names or codes for metabolites of fosetyl-Al. In this summary, a single name or a single code is used for each metabolite. A full list containing structural formula, various names, short forms, codes and occurrences of metabolites is provided as Document N3.

As some pragmatic approach "phosphonic acid" formed as a major metabolite is reported in this Supplementary Dossier as the free acid for the sake of clarity and unequivocal handling. After application, aluminium tris-O-ethyl phosphonate (i.e. fosetyl-Al) dissociates into the O-ethyl phosphonate and aluminium ions. Any phosphonate formed from O-ethyl phosphonate in the following would never be present in the form of the free acid (i.e. phosphonic acid) under the conditions of the environment (pH 4 to 9). This conclusion is supported by the molecular structure and by the dissociation constant observed (dissociation constant for the first step of deprotonation: pKa = 2.0). Consequently, phosphonates in their fully protonated form are strong acids that spontaneously form salts in contact with soil or natural water with any suitable counter ion present (i.e. sodium, potassium, magnesium, calcium). With the ability to readily form salts in the environment phosphonates are, in terms of their acidic or alkaline character, similar to the salts of phosphoric acid (i.e. phosphates) in their environmental behavior. Conclusively, the character of being a salt prevents effects that would be observed when the fully protonated form, i.e. the free acid, would be tested. Consequently, ecotoxicological tests were performed with the sodium or potassium salts rather than with phosphonic acid.

The actual name for metabolite "phosphonic acid" follows current nomenclature according to IUPAC for the free acid H_3PO_3. In the past, i.e. former IUPAC nomenclature, there was used the name phosphorous acid for the same compound. While the actual naming for the salts is "phosphonate" (e.g. disodium phosphonate, Na_2HPO_3) the corresponding former naming was "phosphite" (e.g. disodium phosphite) for this class of compounds.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

Table 8.1- 1: Endpoints birds

Test substance	Test design	Test species	Endpoint			Reference
Fosetyl-Al	acute toxicity	Bobwhite quail	LD_{50}	> 8000 mg a.s./kg bw [a)]		[redacted]; [redacted]; 1981; M-159690-01-1 KCA 8.1.1.1/01
	acute toxicity	Japanese quail	LD_{50}	4997 mg a.s./kg bw		[redacted]; [redacted]; [redacted]; 1977; M-158803-01-1 KCA 8.1.1.1/02
	acute toxicity	Bobwhite quail	LD_{50}	> 2000 mg a.s./kg bw = 3228 mg a.s./kg bw [b)]		[redacted]; [redacted]; 2012; M-444760-01-1 KCA 8.1.1.1/04
	geomean LD_{50}	**Bobwhite quail**	**8000 mg a.s./kg bw**	**5082 mg a.s./kg bw**		**5039 mg a.s./kg bw**
			3228 mg a.s./kg bw			
		Japanese quail	**4997 mg a.s./kg bw**	**4997 mg a.s./kg bw**		
	dietary toxicity (short-term)	Bobwhite quail	LC_{50} LDD_{50}	> 20000 mg a.s./kg diet > 3122 mg a.s./kg bw/d		[redacted]; [redacted]; [redacted]; 1982; M-159687-01-1 KCA 8.1.1.2/01
	dietary toxicity (short-term)	Mallard duck	LC_{50} LDD_{50}	≥ 20000 mg a.s./kg diet > 4616 mg a.s./kg bw/d		[redacted]; [redacted]; [redacted]; 1981; M-159685-01-1 KCA 8.1.1.2/02
	6-weeks feeding chronic, reproduction	Japanese quail	NOEC NOEL	1500 mg a.s./kg diet 216 mg a.s./kg bw/d		[redacted]; [redacted]; [redacted]; 1999; M-189216-01-1 KCA 8.1.1.3/01
	7-weeks feeding chronic, reproduction	Japanese quail	NOEC **NOEL**	≥ 3000 mg a.s./kg diet **≥ 331 mg a.s./kg bw/d**		[redacted]; 2008; M-298080-01-1 KCA 8.1.1.3/02
	LD_{50} / 10	geomean LD_{50}	5039 / 10 = 503.9 mg a.s./kg bw			

Test substance	Test design	Test species	Endpoint		Reference
Phosphonic acid	acute toxicity	Bobwhite quail	LD_{50} LD_{50}	> 2250 mg test item/kg bw > 675 mg pm/kg bw	[redacted]; 1995; M-200039-01-1 KCA 8.1.1.1/03
	dietary toxicity (short-term)	Bobwhite quail	LC_{50} LC_{50} LDD_{50}	> 5620 mg test item/kg diet > 1692 mg pm/kg diet [c] > 508 mg pm/kg bw/d	[redacted]; 1995; M-200041-01-1 KCA 8.1.1.2/03

pm = pure metabolite

[a] 3 mortalities from 10 birds tested at 8000 mg/kg bw, therefore extrapolation factors (EFSA GD 2009; Table 1) not applicable. Included as LD_{50} = 8000 mg/kg bw into the calculation of geomean LD_{50} values

[b] no mortalities among the 5 birds tested at 2000 mg/kg bw, therefore extrapolation factor of 1.614 (EFSA GD 2009; Table 1) applicable: 2000 x 1.614 = 3228 mg/kg bw

[c] Values were corrected for a purity of 41% phosphonic acid weight by volume which is equal to 30.1% weight by weight. Test substance potassium salts of phosphonic acid has a density of 1.36. Therefore, one L of test substance weighs 1360 g and contains 410 g phosphonic acid (410/1360 = 0.301) with a weight/weight purity of 30.1%.

The guideline studies conducted with birds and mammals for use in the reproductive/longterm risk assessments are normally not well suited for ECx calculations. A recent EFSA review assigns a rating of 3 to these guidelines (= "*has serious limitation for the derivation of reliable EC_{10} estimations*") and concludes that "*EC_{10} and EC_{20} and their confidence intervals should not be routinely provided.*"

All studies listed in Table 8.1- 1 are summarized in the following sections. In order to facilitate discrimination between new data and data submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC, the old data (summaries from the original DAR prepared by the RMS) are written in grey typeface.

CA 8.1.1.1 Acute oral toxicity to birds

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

The studies from which the endpoint will be used for risk assessment (directly or for geomean calculation) are summarised below, where applicable based on the evaluations from the original DAR of fosetyl.

Report: KCA 8.1.1.1/01 [redacted]; 1981; M-159690-01-1
Title: The acute oral toxicity (LD50) of LS74.783 to the bobwhite quail
Report No.: R000985
Document No.: M-159690-01-1
Guideline(s): US EPA, Fed. Reg. 163.71-1, 1978
Guideline deviation(s): not specified
GLP/GEP: yes

Methods:
Both range finding and definitive studies were conducted using technical fosetyl-Al (975 g/kg) administered by oral gavage to 5 males and 5 females per dose group, at 5 dose levels in the definitive study (500, 1000, 3000, 5000 and 8000 mg/kg b.w). One control group (10 birds) using corn oil was established.

Results:
Three birds died during the first 3 days following dosing at the highest dose (8000 mg a.s./kg). No post-dosage-mortality occurred in any other groups. Slight effects on bodyweight and behavior (e.g. weakness) were observed at 5000 and 8000 mg a.s./kg bw (recovery at day 4 after dosing).

Findings:
The mortalities observed are presented in the table below:

Dose levels	**mg a.s./kg b.w.**	**0 (control)**	**500**	**1000**	**3000**	**5000**	**8000**
Mortalities	total	0/10	0/10	0/10	0/10	0/10	3/10
	(%)	(0)	(0)	(0)	(0)	(0)	(30)
LD_{50}		**> 8000 mg a.s./kg b.w.**					

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Validity Criteria:
Control mortality not exceeding 10% (fulfilled).

Mean bodyweight [g/bird]:

Test group	**Day 0**	**Day 3**	**Day 7**	**Day 14**
Males				
Control	197	194	196	199
500 mg/kg bw	185	190	192	196
1000 mg/kg bw	186	190	193	196
3000 mg/kg bw	197	194	198	207
5000 mg/kg bw	180	177	181	187
8000 mg/kg bw	183	182	194	202
Females				
Control	182	184	189	194
500 mg/kg bw	177	183	188	191
1000 mg/kg bw	175	186	193	197
3000 mg/kg bw	179	183	191	194
5000 mg/kg bw	183	177	187	194
8000 mg/kg bw	178	171	185	197

Mean food consumption [g/bird]:

Test group	**Day -7 to 0**	**Day 0 to 7**	**Day 7 to 14**
Males			
Control	15	15	18
500 mg/kg bw	13	15	19
1000 mg/kg bw	16	18	19
3000 mg/kg bw	15	14	18
5000 mg/kg bw	13	13	16
8000 mg/kg bw	17	21	21
Females			
Control	16	20	21
500 mg/kg bw	18	21	22
1000 mg/kg bw	15	19	18
3000 mg/kg bw	14	18	18
5000 mg/kg bw	14	16	17
8000 mg/kg bw	19	16	22

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-159690-01-1 KCA 8.1.1.1 /01	EPA71-1 (1978).	OECD 223 (2010)	With OECD TG 223, individual birds are tested per dose whilst the EPA guideline requires 5 males and 5 female birds per dose.	The guideline used in study KCA 8.1.1.1/01 fully satisfies the requirements in OECD TG 223.

Report: KCA 8.1.1.1/02[redacted];[redacted]; 1977; M-158803-01-1
Title: The acute oral toxicity (LD50) of LS 74.783 to the Japanese quail
Report No.: R000524
Document No.: M-158803-01-1
Guideline(s): none
Guideline deviation(s): not applicable
GLP/GEP: no

Methods:
Both a range finding and definitive studies were conducted using technical fosetyl-Al (> 950 g fosetyl-Al/kg). Fosetyl-Al was administered by oral gavage at six dose levels in the definitive study: 4117, 4852, 5259, 6288, 7056 and 8929 mg/kg body weight. Six young adult quail (three males and three females) were allocated to each treatment group. One control using 0.5% sodium carboxymethyl cellulose was established.

Results:
Mortalities were recorded in each group as presented in the table below. With exception of one case at 4852 mg/kg at d 10, all other cases of mortality occurred within the first three days after dosing. The birds became very quiet after dosing, sitting in a hunched position, with feathers ruffled for up to 24 hours. Birds which survived to the end of the study remained in good health throughout the observation period. Food consumption varied between 9 and 18 g/bird/d and there was some evidence that dosing Japanese quail with fosetyl-Al depressed food consumption over the 7-d period after dosing. Moreover, there was no evidence to suggest that body weight was affected.

Findings:
The mortalities observed are presented in the table below:

Dose levels	**mg a.s./kg b.w.**	**0 (control)**	**4117**	**4852**	**5259**	**6288**	**7056**	**8929**
Mortalities	total	0/6	3/6	2/6	3/6	3/6	6/6	5/6
	(%)	(0)	(50)	(33)	(50)	(50)	(100)	(83)
		4997 mg a.s./kg b.w. (95% CI 3 658-6 788 mg a.s./kg b.w.)						

- **Comments (RMS):** This non-GLP study was not conducted according to acceptable guidelines. However, it is scientifically sound. The result is considered as valid.

Further study information supplementing the original DAR summary:

Validity Criteria:
Control mortality not exceeding 10% (fulfilled).

Mean bodyweight [g/bird]:

Test group	**Day 0**	**Day 7**	**Day 14**
Males & females			
Control	192	163	168
4117 mg/kg bw	183	157	135
4852 mg/kg bw	186	154	138
5259 mg/kg bw	200	165	173
6288 mg/kg bw	191	172	192
7056 mg/kg bw	191	-	-
8929 mg/kg bw	168	200	240

Mean food consumption [g/bird]:

Test group	**Day 0 to 7**	**Day 7 to 14**
Males & females		
Control	16	15
4117 mg/kg bw	13	11
4852 mg/kg bw	9	13
5259 mg/kg bw	10	16
6288 mg/kg bw	15	18
7056 mg/kg bw	-	-
8929 mg/kg bw	9	17

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-158803-01-1 KCA 8.1.1.1 /02	EPA71-1 (1978).	OECD 223 (2010)	With OECD TG 223, individual birds are tested per dose whilst the study employed 3 males and 3 female birds per dose.	The guideline used in study KCA 8.1.1.1/02 fully satisfies the requirements in OECD TG 223

Report:	KCA 8.1.1.1/03 [redacted]; [redacted]; 1995; M-200039-01-1
Title:	Potassium salts of phosphorus acid: An acute oral toxicity study with the northern bobwhite
Report No.:	C015193
Document No.:	M-200039-01-1
Guideline(s):	USEPA (=EPA): E 71-1
Guideline deviation(s):	none
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

LD_{50} > 675 mg pm/kg bw

Methods:
Potassium salts of phosphorus acid (*ca* 400 g/L) were administered by oral gavage, at 5 dose levels (0, 292, 486, 810, 1350 and 2250 mg/kg b.w.) with 5 males and 5 females per treatment group.

Results:
At d 3 one male was found dead in the control group. No further mortalities were observed at any treatment level during the test. At d 6 one female at 292 mg/kg exhibited a slight limp. Since this finding was confined to one observation period it was not considered to be treatment-related. At d 0 four birds at 2 250 mg/kg were noted as exhibiting shallow and rapid respiration. Although this observation may be stress-associated with the dosing procedure, it can be considered as a clinical sign since no such observation has been made in the lower dosages. At all later times all birds at 2250 mg/kg were normal in appearance and behaviour. Measured dosages were between 103.3 and 112.6% of nominal values.

LD_{50} > 2250 mg/kg b.w. (> 675 mg H_3PO_3/kg b.w.)
NOEL = 1350 mg/kg b.w. (405 mg H_3PO_3/kg b.w.)

- **Comments (RMS):** This non-GLP study can be used for confirmatory purposes.

Further study information supplementing the original DAR summary:

Validity Criteria:
Control mortality not exceeding 10% (fulfilled).

Mean bodyweight [g/bird]:

Test group	Day 0	Day 3	Day 7	Day 14
Males				
Control	219	218	221	225
292 mg/kg bw	205	209	209	211
486 mg/kg bw	204	207	206	208
810 mg/kg bw	205	209	209	212
1350 mg/kg bw	220	226	227	229
2250 mg/kg bw	211	216	216	216
Females				
Control	219	222	221	218
292 mg/kg bw	222	225	222	223
486 mg/kg bw	204	208	207	210
810 mg/kg bw	220	222	223	222
1350 mg/kg bw	205	209	209	210
2250 mg/kg bw	204	209	208	209

Mean food consumption [g/bird]:

Test group	Day 0-3	Day 4 to 7	Day 8 to 14
Males			
Control	29	30	24
292 mg/kg bw	34	28	21
486 mg/kg bw	36	26	22
810 mg/kg bw	37	26	21
1350 mg/kg bw	18	16	15
2250 mg/kg bw	33	28	21
Females			
Control	29	25	18
292 mg/kg bw	19	13	16
486 mg/kg bw	22	17	17
810 mg/kg bw	25	24	21
1350 mg/kg bw	30	25	19
2250 mg/kg bw	17	17	17

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-200039-01-1 KCA 8.1.1.1 /03	EPA71-1 (1978)	OECD 223 (2010)	With OECD TG 223, individual birds are tested per dose whilst the EPA guideline requires 5 males and 5 female birds per dose.	The guideline used in study KCA 8.1.1.1/03 fully satisfies the requirements in OECD TG 223

One additional study on acute toxicity to birds was performed, which was not submitted for Annex I inclusion on fosetyl under Directive 91/414/EEC and is submitted within this Supplementary Dossier for the approval renewal of fosetyl. This study is summarized below.

Report:	KCA 8.1.1.1/04 [redacted],;[redacted]; 2012; M-444760-01-1
Title:	Toxicity of fosetyl-aluminum (AE F053616) technical during an acute oral LD50 with the northern bobwhite quail (Colinus virginianus)
Report No.:	EBFYL024
Document No.:	M-444760-01-1
Guideline(s):	OPPTS 850.2100 OECD Guideline 223
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Objective:
An acute oral toxicity test was conducted to estimate the LD_{50} of fosetyl-aluminium (fosetyl-Al) technical to northern bobwhite quail (*Colinus virginianus*).

Material and Methods:
Fosetyl-Al, technical; Origin Batch No.: 08001; Batch Code No.: AE F053616-01-11; Tox No.: 09393-00, Purity: 98.1% w/w
Northern Bobwhite quail (24-week-old adults) were orally dosed with Fosetyl-Al technical based on body weight at a limit dose level of 2000 mg a.s./kg body weight. Five birds per dose level (two males and three females) were randomized by body weight into the dose level on experimental Day -1. Birds were capsule dosed on Day -0 and were monitored for 21 days post-dosing. All feed and water was provided *ad libitum*. Adult body weights were taken on experimental Day -1, Day 7, Day 14, and Day 21. Feed consumption was recorded for the first three days of the study and then for Day's 3, 7, 14, and 21. Clinical observations occurred daily. Post-mortem examinations were conducted on all birds sacrificed at study termination.

Dates of experimental work: June 26, 2012 – July 17, 2012

Results:

Acute Oral Toxicity to Birds

Acute Oral LD_{50} With Adult Quail Exposed to Fosetyl-Al Technical	
LD_{50}:	> 2000 mg a.s./kg body weight
Lowest observed adverse effect level (LOAEL):	> 2000 mg a.s./kg body weight
No observed adverse effect level (NOAEL):	2000 mg a.s./kg body weight
Lowest Lethal Dose (LLD):	> 2000 mg a.s./kg body weight

<u>Mortality & Clinical Observations:</u>
No clinical signs of toxicity or mortalities occurred in the control or 2000 mg a.s./kg body weight (bw) dose group. Post-mortem examinations were unremarkable.

<u>Body Weight & Feed Consumption:</u>
There were no statistically significant reductions in body weight or growth at the 2000 mg a.s./kg bw dose level. There were also no dose related reductions in feed consumption at the 2000 mg a.s./kg bw dose level.

Conclusion:
The acute oral LD_{50} for Fosetyl-Al technical in northern bobwhite quail was >2000 mg a.s./kg bw. Based on all parameters, the NOAEL was 2000 mg a.s./kg body weight and the LOAEL was >2000 mg a.s./kg bw.

Applying the appropriate extrapolation factor of 1.614 for a study with no mortality among 5 birds (as given in the EFSA GD 2009, section 2.1.2), the LD_{50} can be established at 3228 mg a.s./kg bw.

CA 8.1.1.2 Short-term dietary toxicity to birds

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. Below the dose conversion calculations for the studies with the active substance are given.

Report:	KCA 8.1.1.2/01 [redacted] T: [redacted]; [redacted]; 1982; M-159687-01-1
Title:	The subacute dietary toxicity (LC50) of LS74-783 to the bobwhite quail
Report No.:	R000984
Document No.:	M-159687-01-1
Guideline(s):	US EPA Fed. Reg., 163.71-2, 1978
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Only one mortality (10%) was observed at the tested level of 20000 ppm of fosetyl-Al. The mean bodyweight of birds over the five day exposure phase at 20000 ppm was 205 g/bird. The mean food consumption during the five day exposure phase at 20000 ppm was 3.2 g/bird/d. The mean achieved daily dietary dose at 20000 ppm is calculated as (3.2/10.5) * 20000 = 3122 mg/kg bw/d.

Conclusion:
The 5-d LD_{50} of fosetyl-Al in bobwhite quails > 3122 mg/kg bw/d.

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Validity Criteria:
Control mortality not exceeding 10% (fulfilled).
Treatment concentrations at least 80% of nominal (fulfilled).
Lowest treatment level without compound-related mortality or other observable toxic effects (fulfilled).

Treatment	**mortality [%]**	**bw day 0 [g]**	**bw day 5 [g]**	**bw day 8 [g]**	**FC day 1-5 [g/bird [a)]]**	**FC day 6-8 [g/bird]**
Control	0	18	26	32	3.6	5
Control	0	17	24	29	3.0	4
Control	10	17	23	29	2.8	4
2353 ppm	0	18	26	34	4.6	4
3361 ppm	0	18	27	34	4.0	3
4802 ppm	0	18	26	32	4.0	4
6860 ppm	0	18	26	33	3.8	4
9800 ppm	0	18	25	30	4.0	4
14000 ppm	0	18	26	32	3.4	4
20000 ppm	10	17	24	30	3.2	4

([a] arithmetic mean of the 5 daily consumption values in the report

General bird health was good throughout the study and no adverse effects were observed in groups dosed with fosetyl-Al during the treatment period.

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-159687-01-1 KCA 8.1.1.2 /01	EPA71-2 (1978)	OECD 205 (1984)	none	Study is no longer required under Regulation (EC) 1107/2009.

Report: KCA 8.1.1.2/02 [redacted]; [redacted]; [redacted]; 1981; M-159685-01-1
Title: The subacute dietary toxicity (LC50) of LS74.783 to the mallard duck
Report No.: R000983
Document No.: M-159685-01-1
Guideline(s): US EPA, Fed. Reg., 163.71-2, 1978
Guideline deviation(s): not specified
GLP/GEP: yes

No mortality was observed up to the top test level of 20000 ppm of fosetyl-Al. The mean bodyweight of birds over the five day exposure phase at 20000 ppm was 204.5 g/bird. The mean food consumption during the five day exposure phase at 20000 ppm was 47.2 g/bird/d. The mean achieved daily dietary dose at 20000 ppm is calculated as (47.2/204.5) x 20000 = 4616 mg/kg bw/d.

Conclusion:
The 5-d LDD_{50} of fosetyl-Al in Mallard duck is > 4616 mg/kg bw/d

❑ **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Validity Criteria:
Control mortality not exceeding 10% (fulfilled).
Treatment concentrations at least 80% of nominal (fulfilled).
Lowest treatment level without compound-related mortality or other observable toxic effects (fulfilled).

Treatment	**mortality [%]**	**bw day 0 [g]**	**bw day 5 [g]**	**bw day 8 [g]**	**FC day 1-5 [g/bird [a)]]**	**FC day 6-8 [g/bird]**
Control 1	0	144	262	318	43.0	47
Control 1	0	141	239	313	40.8	48
Control 3	0	142	243	321	43.4	48
2353 ppm	0	141	250	314	42.6	49
3361 ppm	0	150	278	349	49.2	45
4802 ppm	0	139	254	331	44.6	52
6860 ppm	0	147	259	325	55.4 [a)]	50
9800 ppm	0	145	258	322	49.0	49
14000 ppm	0	143	254	316	49.6	49
20000 ppm	0	148	261	329	47.2	49

([a] mean including an outlier of 90 g on day 1, supposed to be due to spillage

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-159685-01-1 KCA 8.1.1.2 /02	EPA71-2 (1978)	OECD 205 (1984)	none	Study is no longer required under Regulation (EC) 1107/2009.

Report: KCA 8.1.1.2/03 [redacted], [redacted]; [redacted]; 1995, M-200041-01-1
Title: Potassium salts of phosphorus acid: A dietary LC50 study with the northern bobwhite
Report No.: C015194
Document No.: M-200041-01-1
Guideline(s): ASTM: E857-87; OECD: 205; USEPA (=EPA): E71-2
Guideline deviation(s): not specified
GLP/GEP: yes

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

LC_{50} > 1692 mg/kg feed (equ. to 508 mg pm/kg bw/d)

Methods:
The study was conducted using potassium salts of phosphorus acid (*ca* 400 g phosphorous acid/L). Nominal dietary test concentrations were 562, 1000, 1780, 3160 and 5620 ppm. In addition four control replicates were tested. Ten chicks of the Northern bobwhite quail (10 weeks old; not differentiated by sex) were assigned to each treatment group.

Results:
Mean concentration of the test substance in the diet deviated between 100.9 and 110.1% from nominal. No mortality occurred in the control or in any treatment group. Furthermore, there were no clinical signs of toxicity noted at any of the concentrations tested. All birds were normal in appearance and behaviour throughout the test period. No treatment related effect upon body weight or food consumption appeared at any test concentration.

LC_{50} > 5620 ppm (> 1692 mg H_3PO_3/kg feed)
NOEC = 5620 ppm (1692 mg H_3PO_3/kg feed)

❑ **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Validity Criteria:
Control mortality not exceeding 10% (fulfilled).
Treatment concentrations at least 80% of nominal (fulfilled).
Lowest treatment level without compound-related mortality or other observable toxic effects (fulfilled).

Treatment	mortality [%]	bw day 0 [g]	bw day 5 [g]	bw day 8 [g]	FC day 1-5 [g/bird]	FC day 6-8 [g/bird]
Control	0	20	32	43	7	9
Control	0	21	31	41	8	10
Control	0	21	33	43	9	10
Control	0	20	30	40	11	10
562 ppm	0	20	31	40	10	9
1000 ppm	0	21	32	43	7	9
1780 ppm	0	20	31	40	7	9
3160 ppm	0	20	32	41	7	12
5620 ppm	0	21	32	41	8	9

General bird health was good throughout the study and no adverse effects were observed during the treatment period.

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-200041-01-1 KCA 8.1.1.2 /03	EPA 71-2 (1978)	OECD 205 (1984)	none	Study is no longer required under Regulation (EC) 1107/2009.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

One additional study on chronic reproductive toxicity to birds was performed, which was not submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC and is submitted within this Supplementary Dossier for the fosetyl approval renewal. This study has been conducted at treatment levels up to 3000 ppm, in order to match the exposure calculation approach introduced by the previous EU GD for bird and mammal risk assessment (SANCO/4145/2000; 2002). In the past, this study has been included in national product dossier submissions and is summarised below.

Report:	KCA 8.1.1.3/01[redacted],;[redacted];[redacted]; 1999; M-189216-01-1
Title:	Fosetyl-Aluminium: A reproduction study with the Japanese quail (*Coturnix coturnix* japonica)
Report No.:	R014231
Document No.:	M-189216-01-1
Guideline(s):	OECD: Draft, (1998); Equivalent to US EPA OPPTS Guideline No. 850.2300
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Methods:
The test substance was technical substance (purity: 99.6%). Adult birds (10 weeks old, body weights 131 to 243 g were assigned to three treatment groups (nominal concentrations of 167, 500 and 1500 ppm). A control group (0 ppm) was maintained concurrently with the treatment groups. Each group had 16 birds housed in pairs (1 female + 1 male).

Results:
Measured concentrations of the a.s. in diet were close to nominals (168, 453 and 1370 ppm). There were no treatment-related mortalities in any of the treatment groups. However, five incidental mortalities occurred during the course of the study: four occurred in the 167 ppm treatment group and a single mortality occurred in the 500 ppm treatment group. All mortalities were males, they occurred between the first week and the d 4 of wk 0 of the treatment period. Incidental clinical observations normally associated with penwear or interactions among penmates were observed in the control group and in the 167, 500 and 1500 ppm groups. Except for incidental findings, all surviving birds in the control group and the 3 treatment groups were normal in appearance and behaviour throughout the test.

There were no treatment-related effects upon spleen, liver, testes or reproductive tract weight at any of the concentrations tested. Any differences between the treatment and control groups were not statistically significant. There were no treatment related effects upon adult body weight at the 167, 500 or 1500 ppm test concentrations, and any differences from the control group were not statistically significant at any of the body weight intervals.

Due to excessive wastage by some birds, feed consumption was variable among pens. However, there were no apparent treatment-related effects upon feed consumption at all test concentrations

There were no apparent treatment-related effects upon reproductive performance, egg weights, egg shell strength or thickness and body weights of hatchlings or 14-d old survivors at any of the concentrations tested. Any differences between the control group and the treatment groups were not statistically significant for any of the reproductive parameters measured. For all parameters measured, the treatment groups were comparable to or exceeded the control group.

Table B.9.1.3.1-1: Reproduction data for Japanese quail (treatment period)

Dietary concentration	**0 ppm**	**167 ppm**	**500 ppm**	**1 500 ppm**
Eggs laid	623	515	610	659
Eggs laid per female	52	52	53	54
Eggs damaged	5	3	6	3
Eggs damaged / eggs laid (%)	0	1	2	1
Mean egg shell thickness (mm)	0.222	0.216	0.220	0.221
Eggs set	754	623	720	796
Egg weight (g)	12.9	12.8	11.7	12.0

Dietary concentration	**0 ppm**	**167 ppm**	**500 ppm**	**1 500 ppm**
Viable embryos	727	607	697	757
Viable embryos / eggs set (%)	96	97	97	95
Live 2-wk embryos	727	607	697	757
Live 2-wk embryos / viable embryos (%)	100	100	100	100
Hatchling	672	578	658	715
Hatchling / live 2-wk embryos (%)	93	96	95	95
14-d old survivors	653	567	643	685
14-d old survivors / eggs laid (%)	86	90	90	88
14-d old survivors / hatchling (%)	96	93	98	97
Chick bodyweight at hatchling (g)	8	8	8	8
Chick bodyweight at 14 d (g)	59	60	59	59

* significantly different from the control at $p < 0.05$
** significantly different from the control at $p < 0.01$

NOEC = 1500 ppm

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Validity Criteria:
Measured test substance concentrations within 20% of nominal (fulfilled).
Minimum of 16 breeding pairs that have produced eggs available at the end of the 6-week treatment period (fulfilled).
Additional validity criteria in the OECD TG version from 1984 for Japanese quail (at least 24 14-d survivor per hen, eggshell thickness at least 19 mm) were also fulfilled.

The treatment period was proceeded by a two-week pre-treatment period, during which measurements of adult and reproductive parameters were made. These measurements were treated as covariates in the analysis of post-treatment responses in order to increase the power of the test to detect treatment-related effects.

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-189216-01-1 KCA 8.1.1.3 /01	OECD 206 (draft update 1998)	OECD 206 (1984)	With OECD TG 206 (1984), at least 12 pairs of birds per treatment are exposed over 20 weeks and eggs are collected over at least 8 weeks. With OECD TG 206 (1998) at least 6 weeks of egg laying by 16 pairs of proven breeders are required.	The guideline used in study KCA 8.1.1.3/01 is designed for a higher statistical power of detecting reproductive toxicity than the OECD TG 206 in its version from 1984. The used guideline may not be appropriate for bioaccumulating substances because a steady state concentration may not be achieved sufficiently early in the test. This is not the case for fosetyl-Al, therefore the study can be considered as reliable.

Report: KCA 8.1.1.3/02 [redacted]; 2008; M-298080-01-1
Title: Fosetyl-Al: Effects of a subchronical dietary exposure to japanese quails including effects on reproduction and behaviour
Report No.: BAR/REP 013
Document No.: M-298080-01-1
Guideline(s): OECD Draft Guideline 206 for Testing of Chemicals: "Avian Reproduction Test"; draft version from 04/2000
Guideline deviation(s): The exposure period was extended to 7 weeks, since the egg batch of week 4 was destroyed when being transferred to the incubator, so that the reproductive parameter of this week could not be used for evaluation.
GLP/GEP: yes

Objective:
The aim of the study was to determine effects on reproduction of fosetyl-aluminium (fosetyl-Al) to Japanese quail (*Coturnix coturnix japonica*).

Material and Methods:
Fosetyl-Al, technical; purity: 99.9%, specification (batch No.: OP24550120, CAS. No.: 39148-24-8, LIMS No.: 0717456, Spec. No.: 102000002957, Article No.: 05930170); oral administration in diet under laboratory conditions to pairs of sexually mature Japanese Quail (13 weeks old at start of pre-treatment); exposure of 20 breeding pairs per treatment level at nominal dietary concentrations of 1800, 2325 and 3000 mg a.s./kg diet (ppm) and control; 5 weeks of acclimatisation and stabilisation of sexual maturity; 2 weeks of reproductive activity with untreated food (=pre-treatment period) followed by 7 weeks of exposure. Reproductive data from exposure week 4 were excluded from analysis since a tray of eggs was accidentally dropped and all eggs were damaged. Therefore the study was extended for a 7th week of egg laying under exposure, in order to provide for the 6 egg-laying week data required by the guideline.
Effects on parental birds (behavioural impacts, food consumption, body weight, pathological findings) were recorded as well as reproductive parameters (egg weight, egg laying rate, egg fracture, eggshell thickness, fertility, embryonic viability, hatching success) including a 14-day post-hatch observation of the chicks (behavioural impacts, weight of the hatchlings, 14-day body weight).

For statistical evaluation of possible treatment related effects, the data for testing endpoints were processed as unpaired comparisons of each treatment level with untreated control.
Initially the data were analysed on homogeneous distribution (Kolmogoroff-Smirnov test, $p < 0.05$).
In case the data were homogeneously distributed, they were subjected to an analysis of equal variances (Bartlett's test).
In case of equal variances, subsequent analyses were conducted using parametric techniques (Dunnett's test); otherwise the t-Test for inhomogeneous variances (Bonferoni Test) was used.

Dates of Work:

Study initiation.	2007-07-24
Start of acclimation:	2007-07-30 (start of acclimation)
Start of pre-treatment:	2007-09-17
Start of exposure:	2007-10-01
Sacrifice of adults;	2007-11-19
Test termination:	2007-12-20 (sacrifice of chicks)

Validity Check:
The results can be considered as valid. Adult mortality in the untreated control was 0%. The number of eggs laid per hen per day (0.9) and the mean number of 14-day old survivors (0.64 in the controls complied with the typical range specified in the guideline (0.60 to 0.89 eggs per hen per day; 0.34 to 0.71 14-d survivors per hen per day). Eggshell thickness (0.20 to 0.22 mm) was within the specified range (0.19 to 0.22 mm).
The analysis of the food mixtures in the treatment groups revealed that content, homogeneity and stability were within the defined limits.

Findings:

Subchronic and reproduction toxicity to Japanese quail	
Test substance	Fosetyl-Al, tech. a.s.
Test object	Japanese quail
NOEC for parental toxicity [ppm]	≥ 3000
NOEC for parental toxicity [mg a.s./kg bw/d]	≥ 331
NOEC for reproduction [ppm]	≥ 3000
NOEC for reproduction [mg a.s./kg bw/d]	≥ 331

Parental Toxicity
No parameter for parental toxicity showed any statistically or biologically significant difference between control and treatment groups.

Average Body Weight of Parental Birds						
Treatment [ppm]	**Males [g]**			**Females [g]**		
	Start of pre-treatment phase	End of pre-treatment / start of exposure phase	End of exposure	Start of pre-treatment phase	End of pre-treatment / start of exposure phase	End of exposure
0 (control)	248	247	250	289	292	293
1800	239	239	237	294	303	289
2325	248	248	248	298	309	298
3000	246	246	243	286	291	287

Average Food Consumption of Parental Birds [g/pair/day]				
Study Week	**Control**	**1800 ppm**	**2325 ppm**	**3000 ppm**
pre-treat. week 1	55.7	57.0	56.8	53.9
pre-treat. week 2	57.2	55.0	55.9	54.7
exposure week 1	57.9	57.2	56.9	58.7
exposure week 2	58.2	58.2	57.0	57.8
exposure week 3	58.8	58.4	59.0	58.1
exposure week 4	58.7	58.2	60.0	58.8
exposure week 5	61.1	61.1	61.0	60.1
exposure week 6	60.5	59.7	60.4	58.4
exposure week 7	59.9	61.2	61.6	60.2

Body weight, food consumption and achieved daily dietary dose	**0 (control)**	**1800**	**2325**	**3000**
		ppm		
Mean food consumption during exposure [g/pair/d]	59.3	59.1	59.4	58.9
Bodyweight per pair at start of exposure [g]	540	541	557	537
Bodyweight per pair at end of exposure [g]	543	525	545	530
Mean bodyweight per pair during exposure [g]	541	533	551	534
Daily dose/pair [mg a.s./d]	0	106	138	177
Daily dose [mg a.s./ kg b.w./d]	**0**	**199**	**251**	**331**

Neither mortalities nor behavioural changes or impacts were observed.
Gross necropsy demonstrates that the birds were in good shape at test termination. Small changes (capillary bleeding of the intestine or enlarged gall bladder), and females with plucked head, necks and back were found were found in both the control and the treatment groups. The treated birds were not higher affected. The statistical evaluation of the weight of spleen, liver and testicles in the test groups showed no significant changes related to the control.

Reproduction Toxicity
Despite incidental findings in individual parameter and weeks at the lowest treatment level, no statistically or biologically significant differences were observed between control and treatment groups.

Reproductive Performance per hen per day (weekly means per group, absolute data)										
Parameter	Study group	Pre-treatment		Exposure phase						
		week 1	week 2	week1	week 2	week 3	week 5	week 6	week 7	mean
number of eggs laid / hen/day	0 (control)	0.9	0.9	0.9	0.9	1.0	1.0	0.9	0.9	0.93
	1800 ppm	0.9	1.0	1.0	0.9	1.0	1.0	0.9	0.9	0.95
	2325 ppm	1.0	0.9	1.0	0.9	1.0	1.0	1.0	1.0	0.98
	3000 ppm	0.9	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.92
number of 14 day old survivor/ hen/day	0 (control)	0.5	0.6	0.5	0.6	0.6	0.6	0.5	0.5	0.55
	1800 ppm	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.4	0.53
	2325 ppm	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.57
	3000 ppm	0.5	0.5	0.5	0.6	0.6	0.6	0.6	0.5	0.57

Reproductive Performance per hen per day (weekly means per group, absolute data)										
Parameter	Study group	Pre-treatment		Exposure phase						
		week 1	week 2	week1	week 2	week 3	week 5	week 6	week 7	mean
Egg weight [g]	0 (control)	12.3	12.3	12.2	12.3	12.4	12.5	12.6	12.6	9.3
	1800 ppm	12.2	12.3	12.3	12.4	12.2	12.3	12.2	12.3	9.1
	2325 ppm	12.7	12.8	12.8	12.8	12.7	12.7	12.7	12.8	9.4
	3000 ppm	12.3	12.5	12.6	12.6	12.6	12.7	12.6	12.7	9.3
Hatch weight [g]	0 (control)	9.0	9.1	9.3	9.0	9.3	9.2	9.4	9.5	87.2
	1800 ppm	8.9	9.1	9.3	9.0	9.1	9.2	9.1	9.1	81.8
	2325 ppm	9.3	9.4	9.6	9.3	9.2	9.2	9.4	9.4	87.0
	3000 ppm	9.1	9.4	9.5	9.2	9.4	9.2	9.2	9.4	87.3
14-d survivor weight [g]	0 (control)	88.2	81.3	85.6	91.1	84.2	88.6	87.9	85.5	0.21
	1800 ppm	84.0	78.9	83.3	84.6 *	70.4 *	83.7 *	84.6	84.2	0.21
	2325 ppm	86.6	82.9	87.5	90.9	84.9	87.6	86.2	85.1	0.21
	3000 ppm	87.2	82.9	85.2	90.8	86.9	86.7	87.4	87.0	0.21
Eggshell thickness [mm]	0 (control)	0.21	0.21	0.22	0.21	0.21	0.21	0.20	0.21	9.3
	1800 ppm	0.23	0.23	0.21	0.22 *	0.20	0.20	0.21	0.21	9.1
	2325 ppm	0.22	0.21	0.22	0.21	0.20	0.20	0.21	0.21	9.4
	3000 ppm	0.23	0.22	0.22	0.21	0.20	0.21	0.21	0.21	9.3

* statistically significant (p=0.05) different from untreated control

Reproductive Performance per hen (weekly means per group, absolute data)										
Parameter	Study group	Pre-treatment		Exposure phase						
		week 1	week 2	week1	week 2	week 3	week 5	week 6	week 7	mean
Eggs laid	0 (control)	6.6	6.5	6.4	6.5	6.7	6.8	6.6	6.6	6.6
	1800 ppm	6.6	6.7	6.7	6.5	6.7	6.7	6.5	6.6	6.6
	2325 ppm	6.7	6.4	6.8	6.6	6.7	6.7	6.7	6.7	6.7
	3000 ppm	6.6	6.6	6.6	6.4	6.5	6.5	6.7	6.6	6.6
Eggs cracked	0 (control)	0.3	0.2	0.3	0.0	0.2	0.4	0.2	0.1	0.2
	1800 ppm	0.1	0.0	0.3	0.2	0.1	0.1	0.0	0.0	0.1
	2325 ppm	0.2	0.4	0.1	0.1	0.0	0.2	0.0	0.1	0.1
	3000 ppm	0.7	0.4	0.2	0.2	0.1	0.4	0.6	0.2	0.3
Eggs set	0 (control)	5.8	5.8	5.6	6.0	6.0	6.0	6.0	5.8	5.9
	1800 ppm	6.1	6.2	5.9	5.8	6.2	6.2	6.0	6.0	6.0
	2325 ppm	6.1	5.5	6.2	6.4	6.2	6.1	6.2	6.2	6.2
	3000 ppm	5.5	5.8	6.0	5.7	6.0	5.8	6.0	5.8	5.9
Fertile eggs	0 (control)	5.5	5.6	5.3	5.8	5.7	5.7	5.7	5.6	5.6
	1800 ppm	6.0	6.0	5.8	5.6	6.1	6.1	5.8	5.7	5.9
	2325 ppm	5.8	5.4	5.7	5.6	5.7	5.5	5.8	5.6	5.7
	3000 ppm	5.2	5.6	5.9	5.5	5.9	5.5	5.8	5.6	5.7
Early viable embryos	0 (control)	5.4	5.5	5.1	5.7	5.6	5.6	5.6	5.5	5.5
	1800 ppm	5.9	5.8	5.7	5.6	6.1	6.1	5.6	5.6	5.8
	2325 ppm	5.8	5.3	5.7	5.6	5.6	5.4	5.7	5.5	5.6
	3000 ppm	5.2	5.5	5.6	5.3	5.8	5.4	5.7	5.5	5.6
Late viable embryos	0 (control)	5.4	5.5	5.0	5.7	5.6	5.5	5.5	5.3	5.4
	1800 ppm	5.9	5.8	5.6	5.6	6.0	6.0	5.5	5.6	5.7
	2325 ppm	5.8	5.3	5.7	5.5	5.5	5.4	5.6	5.4	5.5
	3000 ppm	5.2	5.5	5.5	5.3	5.6	5.2	5.7	5.5	5.5

Hatchlings (all)	0 (control)	3.7	4.3	3.7	4.2	4.1	4.2	3.8	3.6	3.9
	1800 ppm	4.5	4.1	4.1	4.2	4.0	4.0	3.8	3.0	3.9
	2325 ppm	4.2	4.1	4.2	4.3	4.0	4.4	3.8	3.9	4.1
	3000 ppm	3.8	4.0	3.8	4.5	4.3	4.4	4.5	3.9	4.2
Hatchlings (healthy)	0 (control)	3.6	4.2	3.7	4.1	4.1	4.1	3.7	3.5	3.9
	1800 ppm	4.4	4.0	3.9	4.2	4.0	4.0	3.8	2.9	3.8
	2325 ppm	4.2	4.0	4.1	4.2	3.9	4.3	3.8	3.9	4.0
	3000 ppm	3.8	4.0	3.8	4.5	4.3	4.2	4.5	3.8	4.2
14-d survivors	0 (control)	3.6	4.2	3.6	4.1	4.1	4.0	3.7	3.4	3.8
	1800 ppm	4.4	4.0	3.9	4.1	4.0	3.8	3.8	2.7	3.7
	2325 ppm	4.2	3.9	4.1	4.1	3.9	4.2	3.7	3.8	4.0
	3000 ppm	3.7	3.7	3.8	4.3	4.2	4.2	4.5	3.7	4.1

Reproductive performance in % of control (relative data, 6-week averages)			
Parameter	1800 ppm	2325 ppm	3000 ppm
Eggs laid / hen / day	102%	105%	99%
14 day old survivor/ hen / day	96%	104%	104%
Eggs laid	100%	102%	100%
Eggs cracked	50%	50%	150%
Eggs set	102%	105%	100%
Fertile eggs	105%	102%	102%
Early viable embryos	105%	102%	102%
Late viable embryos	106%	102%	102%
Hatchlings (all)	100%	105%	108%
Hatchlings (healthy)	100%	105%	111%
Egg weight [g]	98%	101%	100%
Hatch weight [g]	94%	100%	100%
14-d survivor weight [g]	100%	100%	100%
Eggshell thickness [mm]	98%	101%	100%

Conclusion
Based on these findings the NOEC of parental toxicity as well of reproductive toxicity is set to be equal or higher 3000 mg a.s./kg food, which corresponds to a dose of 331 mg a.s./kg body weight.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Table 8.1.2- 1: Endpoints mammals

Test substance	Test design	Test species	Endpoint		Reference
Fosetyl-Al	acute toxicity	Rat	LD_{50}	> 2000 mg a.s./kg bw ≡ 5000 mg a.s./kg bw	KCA 5.2.1/04; M-447270-01-1
	acute toxicity	Mouse	LD_{50}	> 2000 mg a.s./kg bw ≡ 5000 mg a.s./kg bw	KCA 5.2.1/05; M-454114-01-1
	Multigeneration reproduction	Rat	NOAEL	6000 ppm ≡ 720 mg/kg bw/d (females) [a]	Document MCA, Section 5.6 Table 5.6- 1
	Developmental toxicity	Rat	NOAEL	≥ 1000 mg/kg bw/d	
	Developmental toxicity	Rabbit	NOAEL	≥ 500 mg/kg bw/d	
Phosphonic acid	Several studies were carried out for assessing the toxicity of the major metabolite of fosetyl-Al, phosphonic acid (previously referred to as "phosphorous acid") which is present in significant amounts in plants and in and in relevant body compartments of mammalian laboratory animal species. Phosphonic acid and its salts are of low acute toxicity via all routes of administration. Sub-chronic and chronic feeding studies with phosphonates have not revealed any specific effect of concern. Sodium phosphonate was not carcinogenic in a 27-month feeding study in rats Taken together, these data indicate the absence of any critical toxicity of the plant metabolite of fosetyl-Al, phosphonic acid.				Document MCA, Section 5.8.1

[a] justification for refined dose calculation is provided in Section CA 8.1.2.2

CA 8.1.2.1 Acute oral toxicity to mammals

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The conclusion in the DAR on these studies was: "Fosetyl-aluminium was of low oral, dermal and inhalation toxicity: the acute oral LD_{50} in rats was greater than 5000 mg/kg."

New studies conducted in rat (KCA 5.2.1/04, M-447270-01-1) and mice (KCA 5.2.1/05, M-454114-01-1) confirm that fosetyl-aluminium is practically non-toxic in acute exposure of mammals.

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

As a result of the original evaluation for the Annex I inclusion of fosetyl under Directive 91/414/EEC, the endpoint for long-term risk assessment on wild mammals was established at 6000 ppm, the NOAEL from the 3-generation rat reproduction study (KCA 5.6.1/01, M-203019-01-1).

It is proposed to retain the reproductive risk assessment endpoint of 6000 ppm from the rat reproduction study, but to adapt the dose conversion to the recommendations of the EFSA GD (2009).

Table 8.1.2.2- 1: Effects of fosetyl-Al on reproduction

Test substance	Exposure	Species	Endpoint	Reference
Fosetyl-Al	Long-term risk assessment	Rat	NOAEL 6000 ppm ≡ 439 mg a.s./kg bw/d NOAEL 6000 ppm ≡ 720 mg/kg bw/d	M-203019-01-1 KCA 5.6.1/01

A good overview on the results of this study is given in the summary in the original DAR, notably in Table 6.6.1- 5.Litter data.

In this study, 3 generations of rat were kept under continuous dosing via diet to fosetyl-aluminium (fosetyl-Al) at concentrations of 6000, 12000 and 24000 ppm over nearly 2 years (91 weeks). During that time, 6 litters were produced (F1A, F1B, F2A, F2B, F3A and F3B).

Over this prolonged exposure to very high concentrations, fosetyl-Al did no produce any adverse effects on reproductive performance and fertility in rats over 3 generations. The NOAEL for reproductive performance or fertility was 24000 ppm (equivalent to 1782 and 1997 mg/kg bw in males and females of the F0 generation, respectively), as no treatment- related effects were observed in any generation.

Most notable with regard to effects of relevance for the ecotoxicological risk assessment, a statistically significant lower body weight of pups was observed in all six litters of all three generations in the 21-d pup weight and/or 21-d litter weight at 24000 ppm.

Only in the F2B generation, this effect on 21-d pup weight was also statistically significant at 12000 ppm. Therefore the NOAEL for development was set at 6000 ppm based on these effects on body weight of F2B generation in the 12000 ppm treated group.

This NOAEL of 6000 ppm in the F2B generation had been converted into a dose of 439 mg/kg bw/d which has been achieved by the parental males (F0) during the first pre-mating phase before yielding the F1A litter.

However, the achieved dose of the parental males (F0) siring the F1A litter has little if any bearing on the substance intake by sires of the F2B litter:

1. The LOAEL on 21-d pup and litter weight of the F1A is 24000 ppm, so if the pre-mating dose of parental males (F0) should be selected for risk assessment, it should be the dose achieved by these parental males at 12000 ppm (865 mg/kg bw/d).
   This is however not recommended because there is no causal link between the achieved pre-mating dose of males at any generation, and the effect on pup and/or litter weight occurring during the respective lactation phase of the pups.

2. It is more plausible to link this effect on pup and/or litter weight occurring during the respective lactation phase to the exposure of the lactating mothers.
   Thus, the dose conversion of the NOAEL at 6000 ppm should be accomplished based on body weight and food consumption of the lactating <u>females</u> rather than of the males.

3. Given that the statistically significant effect at 12000 ppm was established only in the F2B pups, the most appropriate data for dose conversion would be body weight and food consumption of the mothers of the F2B pups during that lactation phase.

4. However, food consumption during the lactation phases is not reported in M-203019-01-1 (only during the different pre-mating phases).
   Based on these data, the dose associated with 6000 ppm for females from the pre-mating phase <u>prior</u> to littering and nursing the F2B pups is 657.3 mg/kg bw/d.

Achieved dose [mg/kg bw/d] of females during the 3 pre-mating phases of the rat reproduction study with fosetyl-Al (based on Table 1 of the original report M-203019-01-1, page 31)

Females	week	1	2	3	4	5	6	7	8	9	10	11	12	13	mean
6000 ppm	F0	806	679	622	577	550	519	476	469	416	429	436	401	374	519.5
	F1B	896	893	776	674	570	478	445	477	444	396				604.9
	F2B	1161	1026	863	805	731	640	592	568	544	459	378	395	383	**657.3**
12000 ppm	F0	1582	1310	1190	1149	1103	963	928	878	807	808	807	738	759	1001.7
	F1B	1796	1745	1544	1247	1092	949	889	924	898	747				1183.1
	F2B	2262	1914	1714	1629	1387	1258	1153	1096	1054	859	720	773	753	1274.8
24000 ppm	F0	3112	2585	2378	2203	2150	2032	2032	1711	1591	1665	1635	1510	1542	2011.2
	F1B	4331	4528	3599	2839	2454	2107	2107	2106	1957	1856				2788.4
	F2B	4944	4277	3566	3336	2988	2762	2762	2451	2222	1878	1525	1655	1618	2768.0

5. The EFSA GD 2009 however specifies in section 2.3.1.1 ("*Conversion of endpoints from ppm to mg a.s./kg bw/d*") that "*it is more appropriate to use the consumption relevant to the specific reproductive phase*".
   Thus, the dose during the premating phase is not fully appropriate for effects during the lactation phase, but the dose during lactation is not reported.
   Therefore the following instructions in that section of the EFSA GD 2009 apply in this case:

Table 2 presents a standard set of factors that can be used to provide internal consistency when converting concentrations in diet into mg/kg bw/d dose levels for mammals. This should be used only in the absence of specific information in a study report or summary (it can, however, be used to give a rough check of values cited in a study). Only routine study types, species and ages have been considered.

Table 2. Factors for converting endpoints from mammalian toxicity studies from ppm to mg a.s./kg bw/d. Endpoints reported as ppm should be multiplied by the relevant factor from the table to convert them to mg/kg bw/d.

Species	Age/study	Conversion factor from ppm to mg/kg bw/d
Rat	28 d and 90 d	0.1
Rat	Two-generation study first mating*	0.08
Rat	Two-generation study overall (females)*	0.12
Mouse	28 d and 90 d	0.20
Dog	adult/all	0.025

*The first mating value for a two-generation study should be used for assessment when effects (general or on reproduction) are seen to relate to the pre-mating phase of the first mating of a study, or effects seen only in male F0 parents at any time. For all other aspects of a two-generation study the overall conversion figure should be used.

(Screenshot of EFSA GD 2009: Risk Assessment for Birds and Mammals)

6. The dose achieved by the lactating mothers during the lactation phase of the F2B pups can therefore be estimated with the generic conversion factor of 0.12 provided in the EFSA GD 2009 (section 2.3.1.1 Conversion of endpoints from ppm to mg a.s./kg bw/d).
   With that factor of 0.012 applied on the dietary concentration of 6000 ppm, the NOAEL is calculated as 6000 x 0.12 = 720 mg/kg bw/d.

⇨ **Therefore, the dietary concentration of 6000 ppm is equivalent to a NOAEL of 720 mg/kg bw/d. This NOAEL is the appropriate endpoint for reproductive risk assessment on wild mammals for fosetyl-Al.**

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

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds 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 the active substance fosetyl-aluminium and its metabolite is below the trigger (<3), no evaluation of secondary poisoning is needed (see Document MCP, Section 10.1.1 for more details).

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

Information on effects of fosetyl-aluminium on reptiles or amphibians is not available. Risk to birds and mammals is assessed in Document MCP, Section 10.1.

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 protocol is available, this test was developed to evaluate to potential effect on the thyroid system and not to measure population relevant effects.

A literature review was carried out for fosetyl according to the requirements of the Regulation (EU) No 844/2012, which itself refers to Article 8(5) of Regulation (EC) No 1107/2009. The review itself is in accordance with the EFSA Guidance Document. No public literature at all was found that could have been evaluated for fosetyl-Al effects on amphibians or reptiles.

Therefore no further studies can be suggested at this time for these groups of organisms.

CA 8.1.5 Endocrine disrupting properties

Wild Mammals
Designated studies on endocrine disrupting (ED) properties of fosetyl-aluminium (fosetyl-Al) have not been conducted by the applicant. The existing body of data is sufficient to exclude relevant ED-like potential of fosetyl-Al. This is based on the absence of effects on the histopathology or weight of reproductive organs, thyroids and pituitary. In addition, the available fertility studies showed no effects on male or female fertility, which may be considered sensitive targets of ED-like activity. Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of fosetyl-Al in mammals.

Birds
The population relevant effects of fosetyl-Al on birds were studied in a 6-week reproductive toxicity study on Japanese quail. No statistically significant effects on adult birds, offspring or reproductive parameters were found at the highest tested dose level of 3000 mg fosetyl-Al/kg diet.
As reproduction was not affected in this avian species even at very high dose levels, it is concluded that there are no population relevant adverse effects of fosetyl-Al.
Based on the absence of any indication of relevant effects it can be concluded that fosetyl-Al is not a (potential) endocrine disrupter.
No further testing for endocrine disrupting properties is warranted.

CA 8.2 Effects on aquatic organisms

For studies already evaluated during the last EU review of fosetyl-Al, please refer to the corresponding section in the DAR and to the studies in the baseline dossier provided by Bayer CropScience.
In order to complete the aquatic risk assessment and to address new data requirements according to Regulation (EC) No 1107/2009, additional studies were performed and are provided within this Supplemental Dossier for approval renewal of fosetyl.

Table 8.2- 1: Endpoints used in risk assessment and additional studies for fosetyl-Al and its metabolite

Test substance	Test species	Endpoint		Reference
Fosetyl-Al	Fish, acute *Lepomis macrochirus*	LC_{50}	> 60 mg a.s./L (mm)	[redacted]; 1997; M-184477-01-1 KCA 8.2.1/02
	Fish, acute *Oncorhynchus mykiss*	LC_{50}	> 122 mg a.s./L (mm)	[redacted].; 1999; M-189219-01-1 KCA 8.2.1/04
	Fish, acute *Cyprinus carpio*	LC_{50}	> 100 mg a.s./L (nom)	[redacted] L; 2013; M-449083-01-1 KCA 8.2.1/05
	Fish, chronic *Oncorhynchus mykiss*	NOEC	≥ 100 mg a.s./L (nom)	[redacted]; 1997; M-184572-01-1 KCA 8.2.2/01
	Fish, chronic *Pimephales promelas*	NOEC	0.213 mg a.s./L (nom)	[redacted].; [redacted]; 2015; M-531353-01-1 KCA 8.2.2.1/01
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 100 mg a.s./L (nom)	[redacted].; 1996; M-170914-01-1 KCA 8.2.4.1/01
	Invertebrate, chronic *Daphnia magna*	NOEC	17 mg a.s./L (mm)	[redacted].; 1996; M-189214-01-1 KCA 8.2.5.1/01
	Algae *Desmodesmus subspicatus* (*Scenedesmus subspicatus*, green algae)	E_bC_{50} E_rC_{50}	5.9 mg a.s./L (mm) > 16 mg a.s./L (mm)	[redacted].; 1999; M-189220-01-1 KCA 8.2.6.1/01
	Algae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*, green algae)	7d-E_dC_{50} 72h-E_rC_{50}	4.99 mg a.s./L (mm) recalculation 9.54 mg a.s./L (mm)	[redacted] P; 1989; M-163526-01-1 KCA 8.2.6.1/03 [redacted]; 2005; M-253825-01-1 KCA 8.2.6.1/04
	Algae *Desmodesmus subspicatus* (*Scenedesmus subspicatus*, green algae)	E_bC_{50} E_rC_{50}	24.9 mg a.s./L (nom) 43.3 mg a.s./L (nom)	[redacted]; 2007; M-289324-01-1 KCA 8.2.6.1/05
	Algae *Navicula pelliculosa* (diatom)	7d-E_dC_{50} 72h-E_rC_{50}	8.93 mg a.s./L (mm) recalculation 18.11 mg a.s./L (mm)	[redacted].; 1988; M-163531-01-1 KCA 8.2.6.2/01
	Aquatic plant *Lemna gibba*	14d-E_yC_{50} 7d-E_rC_{50}	79.67 mg a.s./L (mm) recalculation: 166.6 mg a.s./L (mm)	[redacted] F; 1989; M-163537-02-1 KCA 8.2.7/01 [redacted].; 2015; M-525565-01-1 KCA 8.2.7/02

Test substance	Test species	Endpoint		Reference
Phosphonic acid	Fish, acute, *Oncorhynchus mykiss*	LC_{50}	> 28.6 mg pm/L (mm) [a)]	[redacted]; 1994; M-179069-01-1 KCA 8.2.1/03
	Fish, acute, *Oncorhynchus mykiss*	LC_{50}	> 400 mg pm/L (nom)	[redacted]; 2008; M-310496-01-1 KCA 8.2.1/06
	Fish, acute *Lepomis macrochirus*	LC_{50}	> 35.7 mg pm/L (nom) [b)]	[redacted]; 1999; M-171840-01-1 KCA 8.2.1/04
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 29.0 mg pm/L (mm) [a)]	[redacted]; 1994; M-179068-01-1 KCA 8.2.4.1/02
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 400 mg pm/L (nom)	[redacted]; 2008; M-310318-01-1 KCA 8.2.4.1/03
	Sediment dweller *Chironomus riparius*	NOEC	> 100.2 mg pm/L (nom) [b)]	[redacted]; 1999; M-171912-01-1 KCA 8.2.5.4/01
	Algae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*, green algae)	E_bC_{50} E_rC_{50}	8.6 mg pm/L (nom) [b)] 29.4 mg pm/L (nom) [b)]	[redacted]; 1999; M-171844-01-1 KCA 8.2.6.1/02

a.s. = active substance, pm = pure metabolite
mm = mean measured; nom = nominal

[a)] Values were corrected for a purity of 41% phosphonic acid weight by volume which is equal to 29.7% weight by weight. Test substance potassium salts of phosphonic acid has a density of 1.38. Therefore, one L of test substance weighs 1380 g and contains 410 g phosphonic acid (410/1380 = 0.297) with a weight/weight purity of 29.7%.

[b)] Values were corrected for a purity of 40.9% phosphonic acid weight by volume which is equal to 29.7% weight by weight. Test substance potassium salts of phosphonic acid has a density of 1.376. Therefore, one L of test substance weighs 1376 g and contains 409 g phosphonic acid (409/1376 = 0.297) with a weight/weight purity of 29.7%.

Recalculations of chronic EC_{10} values are provided in the table below for both fosetyl-Al and its metabolite phosphonic acid.

Chronic EC_{10} values (and 95% Confidence Limits) of fosetyl-Al (a.s.) and phosphonic acid for aquatic organisms.

Test substance	Test species	Endpoint		Reference
Fosetyl-Al (a.s.)	Fish, chronic *Oncorhynchus mykiss*	EC_{10} 95% CL	nd* nd	[redacted]; 1997; M-184572-01-1 KCA 8.2.2/01
	Fish, chronic *Pimephales promelas*	EC_{10} 95% CL	0.31 mg a.s./L (nom) 0.05 – 0.56	[redacted]; [redacted]; 2015; M-531353-01-1 KCA 8.2.2.1/01
	Invertebrate, chronic *Daphnia magna*	EC_{10} 95% CL	17.7 mg a.s./L (mm) nd**	[redacted]; 1996; M-189214-01-1 KCA 8.2.5.1/01
	Algae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*), green algae	72-h E_rC_{10} 95% CL	3.40 mg a.s./L (mm) 2.63 – 4.06	[redacted]; 2005; M-253825-01-1 KCA 8.2.6.1/03
	Algae *Desmodesmus subspicatus* (*Scenedesmus subspicatus*), green algae	72-h E_rC_{10} 95% CL	18.63 mg a.s./L (nom) 9.33 – 26.71	[redacted]; 2007; M-289324-01-1 KCA 8.2.6.1/04
	Algae *Navicula pelliculosa* (diatom)	72-h E_rC_{10} 95% CL	7.07 mg a.s./L (mm) 3.24 – 9.78	[redacted]F; 1988; M-163531-01-1 KCA 8.2.6.2/01
	Aquatic plant *Lemna gibba*	7-d E_rC_{10} 95% CL	65.23 mg a.s./L (mm) 31.67 – 89.69	[redacted]; 2015; M-525565-01-1 KCA 8.2.7/02
Phosphonic acid	Sediment dweller *Chironomus riparius*	EC_{10} 95% CL	nd*** nd	[redacted]; 1999; M-171912-01-1 KCA 8.2.5.4/01
	Algae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*), green algae	72-h E_rC_{10} 95% CL	13.14 mg pm/L (nom) 9.04 – 17.30	[redacted]; 1999; M-171844-01-1 KCA 8.2.6.1/02

a.s. = active substance; mm = mean measured; nom = nominal; nd: not determined
* Over the test period, growth rate inhibition never exceeded 9% for the range of concentrations tested (10, 18, 32, 56 and 100 mg/L).
** No statistically significant differences between the controls and the 1.0, 3.2, 10 and 32 mg/L test groups for the number of juveniles produced per adult after 14 and 21 days.
*** No statistically significant differences between control and treated conditions, including the highest concentration tested (338 mg/L), for both the emergence and development rate.

All studies listed in Table 8.2- 1 are summarized in the following sections. In order to facilitate discrimination between new data and data submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC, the old data (summaries from the original DAR prepared by the RMS) are written in grey typeface.

CA 8.2.1 Acute toxicity to fish

For more information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

Two additional studies on acute toxicity to fish were performed, which were not submitted for Annex I inclusion of fosetyl under Directive 91/414/EEC and are submitted within this Supplementary Dossier for the approval renewal of fosetyl.

Report:	KCA 8.2.1/01 [redacted]; 1999; M-189219-01-1
Title:	Fosetyl-Al: Acute toxicity for rainbow trout (*Oncorhynchus mykiss*)
Report No.:	R014234
Document No.:	M-189219-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, Part C, Method 1; OECD: 203; Equivalent to US EPA OPPTS Guideline No. 850.1075
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Methods:
The experimental design (semi-static limit test with daily renewal) included two experimental groups with 2 replicates per group and 10 fish per replicate. The experimental groups were a dilution water control and one nominal concentration of fosetyl-Al (120 mg/L; purity: 978 g/kg).

Results:
The test substance was soluble in the dilution water at the concentration tested. Measured concentrations ranged from 101 to 103% of nominal values at 0 h, from 103 to 104% of nominal at 24 h, from 99 to 101% of nominal at 72 h and from 98 to 100% of nominal at 96 h. No mortality or sublethal toxicity was observed in the control groups or in the two groups of ten fish exposed for 96 h to a mean measured concentration of 122 mg/L of fosetyl-Al.

Conclusions:
LC50 - 96 h > 122 mg a.s./L (mean measured concentration)
NOEC - 96 h = 122 mg a.s./L (mean measured concentration)

- **Comments (IVMS):** acceptable

Further study information supplementing the original DAR summary:

Objective:
The aim of the study was to assess the acute toxicity of fosetyl-Al to rainbow trout *(Oncorhynchus mykiss)*, expressed as 96h-LC_{50} for mortality, under semi-static conditions.

Materials and methods:
Test item: Fosetyl-Al, purity: 978 g/kg, Lot No.: OP9950059
Test was conducted over a period of 96 hours with *Oncorhynchus mykiss* in softened, dechlorinated, filtered laboratory tap water with a chlorine level from 0.02 to 0.09 mg/L and a hardness level of 132 to 168 mg $CaCO_3$/L. A photoperiod of 16 hours light : 8 hours dark was maintained. Fish were not fed during the 96 hours exposure period. The mean standard length was 3.8 cm and the mean body weight was 0.86 g, corresponding to a loading of 0.43 g fish tissue/L.
Concentrations of test substance were measured at 0 and 72 hours (fresh media) and 24 and 96 hours (expired media) by chemical analysis. The parameters pH, dissolved oxygen and temperature were measured daily.

Records for any mortalities or incidences of sublethal effects in the fish were made at 0.25, 2, 4, 24, 48, 72 and 96 hours during the test.

Findings:

Validity criteria:

Validity criteria (according to OECD 203, adopted 17.07.1992)	**Obtained in this study**
Mortality in the controls (criterion is < 10%)	0%
Dissolved oxygen concentration in the control and test vessels (criterion is ≥ 60%)	> 60%

Temperature was 14 to 15 °C, *i.e.*, in the recommended range of 13 to 17 °C

Analytical findings:
All results are expressed in terms of mean measured concentration. Measured concentrations ranged from 101 to 103% of nominal at 0 hours, 103 to 104% of nominal at 24 hours, 99 to 104% of nominal at 72 hours and 98 to 100% of nominal at 96 hours.
The pH values in the test ranged from 7.2 to 8.1, dissolved oxygen concentrations ranged from 7.5 to 10.3 mg/L and temperature ranged from 14 to 15 °C.

Conclusion
The acute toxicity of fosetyl-Al to *Oncorhynchus mykiss* was investigated and gave the 96-hour LC_{50} of > 122 mg/L (based upon mean measured concentrations). The no observed effect concentration (NOEC) was 122 mg/L based on the lack of mortality and sublethal effects at this test concentration.

Report:	KCA 8.2.1/02 [redacted]; 1997; M-184477-01-1
Title:	Fosetyl-Al: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*).
Report No.:	R011943
Document No.:	M-184477-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, C1; OECD: 203, (1992); Equivalent to US EPA OPPTS Guideline No. 850.1075
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The aim of the study was to determine the acute toxicity of fosetyl-Al to bluegill sunfish *(Lepomis macrochirus)*, expressed as 96h-LC_{50} for mortality.

Materials and Methods:
Test item: Fosetyl-Al (tech.), analyzed content of active substance: fosetyl-Al: 970 g/kg, specified by batch no. OP9607181.
Test organism: Bluegill sunfish *(Lepomis macrochirus)*, mean body length 4.7 cm, mean body weight 1.12 g. The biomass loading for this test was 0.56 g fish tissue/L test medium.
At the start of the study, 10 fish were placed in each test vessel at random, in the prepared test solutions for 96 h under semi-static test conditions to a nominal (mean measured) concentration of 100 (60) mg fosetyl-Al/L against a water control.
The test vessels were then covered to reduce evaporation and maintained at 21°C in a temperature-controlled room with a photoperiod of 16 hours light and 8 hours darkness with a 20 minute dawn and dusk transition period for 96 hours. The test vessels were aerated via narrow bore glass tubes. The fish were not individually identified and received no food during exposure.
The number of mortalities and any sub-lethal effects of exposure in each test and control vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the study until termination after 96 hours.
The endpoints were expressed as mean measured concentrations.

Dates of experimental work: August 04, 1997 to August 08, 1997
Findings:

Validity criteria:

Validity Criteria	Recommended	Obtained
Mortality in the control	≤ 10%	0%
Constant water quality and environmental conditions during the test	Yes	Yes
Concentration of dissolved oxygen	≥ 60%	≥ 60%

All validity criteria for the study were met.

Analytical results:
Analysis of the freshly prepared test preparations at 0 hours showed the measured concentrations to be 92 and 93% of nominal for replicates 1 and 2 respectively.
At 24 and 96 hours, analysis of the 24-hour old unstirred media gave measured concentrations of between 33 and 39% of nominal. However, analysis of the stirred test media showed measured concentrations between 94 and 98% of nominal.
Given the low measured concentrations obtained for the unstirred test media it was considered justifiable to base the results in terms of the time-weighted mean measured concentration calculated for the initial 24 hour dosing period. The samples of unstirred media taken at 24 and 96 hours showed very similar results and therefore the 0-24 hour time-weighted mean measured concentration is considered to be representative of the exposure level for the whole of the study period.

Biological results:
There were no mortalities or sub-lethal effects in 20 fish exposed to a test concentration of 100 mg/L for a period of 96 hours.

LC_{50} values for bluegill sunfish to fosetyl-Al based on nominal concentrations

Test substance:	Fosetyl-Al
Test object:	Bluegill sunfish *(Lepomis macrochirus)*
Exposure:	96 hours, static test design (limit)
LC_{50} 96 h:	**> 60 mg (mean measured) test item / L**

Conclusions:
The 96-hour LC_{50} based on the time-weighted mean measured concentrations was greater than 60 mg/L and correspondingly the No-Observed Effect Concentration was greater than or equal to 60 mg/L.

- **Comments (RMS):** acceptable

The summary presented above from the original DAR was already presented with further results and details for the submission of the fosetyl EU AR Supplementary Dossier.

Further study information supplementing the original DAR summary:

Materials and methods:
Following a preliminary range-finding study, acute bioassay was conducted as a limit test in a semi static test design. Media used in test was laboratory tap water dechlorinated by passage through an activated carbon filter and partly softened giving water with a hardness of approximately 100 mg/L as $CaCO_3$.

Analysis of the test preparations at 0, 24 and 96 hours was carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES) for the aluminium concentration. Afterwards, the aluminium concentration was converted into a concentration of test material by multiplying by the appropriate factor.
The water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the study.

Findings:

Validity criteria:
Temperature was 21 °C, *i.e.*, in the recommended range of 21 to 25 °C.

Physico-chemical findings:
The pH values in the test ranged from 6.8 to 7.7, dissolved oxygen concentrations ranged from 8.3 to 8.8 mg/L and water temperature was 21 C.

Report:	KCA 8.2.1/03 [redacted], [redacted]; 1994; M-179069-01-1
Title:	Potassium salts of phosphorous acid: Acute toxicity to rainbow trout, *Oncorhynchus mykiss*, under static test conditions.
Report No.:	R009323
Document No.:	M-179069-01-1
Guideline(s):	USEPA (=EPA): E, 154-8
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Methods:
Ten juvenile rainbow trout were randomly assigned to the nominal test concentration of 100 mg/L (ca 400 g phosphorus acid/L) and control treatment (dilution water). Ten fish were added to each test container and all treatments triplicated, resulting in 30 fish per treatment. Fosetyl-Al: 678 g/kg of test substance.

Results:
Potassium salts of phosphorous acid test concentrations remained relatively stable throughout the 96-h static exposure. The mean measured concentration of potassium salts of phosphorous acid was 96.4 mg/L which was 96% of nominal. Mortality of rainbow trout exposed for 96 h to potassium salts of phosphorous acid was 3%. A mortality of 3% was also observed in the dilution water control. No sublethal effect was noted during the test.

Conclusions:
LC50 - 96 h > 96.4 mg/L (mean measured concentrations) (> 28.6 mg H_3PO_3/L)
NOEC - 96 h = 96.4 mg/L (mean measured concentrations) (= 28.6 mg H_3PO_3/L)

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary

Objective:
The aim of the study was to determine the acute toxicity of potassium salts of phosphorous acid to rainbow trout (*Oncorhynchus mykiss*), expressed as 96h-LC_{50} for mortality, under static conditions.

Materials and methods:
Test item: Potassium salt of phosphorous acid containing 410 g/L phosphorus acid, Batch No.: 224-3, Lot No.: CSI 64-F5B.
Test was conducted over a period of 96 hours in a static system with three replicate test vessels and three control vessels. Concentrations of test substance were measured at 0 and 96 hours by a titrimetric analysis followed by LTM-017 method. The parameters pH, dissolved oxygen, and temperature were measured daily. Dilution water was a moderately hard freshwater. At test initiation, water possessed a hardness of 70 mg/L as calcium carbonate, alkalinity of 22 mg/L as calcium carbonate and a specific conductivity of 377 µS/cm.
Within the 48 hours prior to the test and during the whole test, fish (*Oncorhynchus mykiss*) were not fed. The range of individual fish length and weight measured after 96 hours was 31 to 43 mm (mean = 36.9 mm) and 0.36 to 1.14 g (mean = 0.69 g), respectively. Loading was calculated to be 0.23 g fish tissue/L of test solution. Records for any mortalities or symptoms of toxicity in the fish were made at 24, 48, 72 and 96 hours during the test.

Findings:

Validity criteria:

Validity criteria (according to OECD 203, adopted 17.07.1992)	**Obtained in this study**
Mortality in the controls (criterion is < 10%)	0%
Dissolved oxygen concentration in the control and test vessels (criterion is ≥ 60%)	≥ 60%

Temperature was within 21.7 to 21.9 °C, *i.e.*, in the recommended range of 21 to 25 °C, and fish had a mean length of 30 mm, in the recommended length of 20 ± 10 mm.

Analytical findings:
The pH values in the test ranged from 6.5 to 7.6, dissolved oxygen concentrations ranged from 7.9 to 11.3 mg/L, total hardness of the dilution water was 68 to 70 mg/L, conductivity of the water was 377 to 390 µS/cm and temperature ranged from 11.9 to 12.6 °C.

Biological findings:

Mean measured concentration (mg/L)	**Cumulative Number dead fish (percent mortality)**			
	24 hour	48 hour	72 hour	96 hour
Control	0 (0)	0 (0)	1 (3)	1 (3)
96.4	0 (0)	0 (0)	0 (0)	1 (3)

Conclusion
The acute toxicity of potassium salt of phosphorous acid to *Oncorhynchus mykiss* has been investigated and gave the 96-hour LC_{50} of > 96.4 mg/L (based upon mean measured concentrations). The no observed effect concentration (NOEC) was 96.4 mg/L based on the lack of mortality and sublethal effects at this test concentration.

Report:	KCA 8.2.1/04 [redacted]; 1999; M-171840-01-1
Title:	EXP10679A (Potassium salt of phosphorous acid): Determination of acute toxicity to bluegill sunfish (*Lepomis macrochirus*)
Report No.:	R005931
Document No.:	M-171840-01-1
Guideline(s):	OECD: 203, (1992); USEPA (=EPA): 540/9-85-006; OPPTS 850.1075
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Methods:

There were two experimental groups with 3 replicates/group and 10 animals per replicate. The experimental groups included a dilution water control and a nominal test concentration of 120 mg/L (purity: 409 g phosphorous acid/L). The definitive test was conducted as a limit test with one single test substance concentration because during range-finding work (two nominal test substance concentrations of 32 and 180 mg/L) no toxic effects were observed.

Results:

At test initiation, analytical measurements showed the measured concentrations were 100% of the nominal value of 120 mg/L. The measured concentrations after 72 h were 130 mg/L (108% of nominal). The nominal concentration was used for the calculation of toxicity values. No mortality or sublethal toxicity was observed in the control or test substance groups over the 96-h exposure period.

Conclusions:

LC_{50} - 96 h > 120 mg/L (nominal concentrations) (> 35.7 mg H_3PO_3/L)
NOEC - 96 h = 120 mg/L (nominal concentrations) (= 35.7 mg H_3PO_3/L)

❑ **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Objective:

The aim of the study was to determine the acute toxicity of potassium salts of phosphorous acid to bluegill sunfish (*Lepomis macrochirus*), expressed as 96h-LC_{50} for mortality, under flow-through conditions.

Materials and methods:

Test item: Potassium salt of phosphorous acid containing 409 g/L phosphorus acid.
Test was conducted over a period of 96 hours in a flow-through system with three replicate test vessels and three control vessels. Test solutions were renewed at a nominal rate of 250 mL/min to produce 6.7 volume additions per 24 hours. Concentrations of test substance were measured at 0 and 96 hours by a titrimetric method. The parameters pH dissolved oxygen and temperature were measured daily.
During the whole test, fish (*Lepomis macrochirus*) were not fed. The range of individual fish length and weight measured after 96 hours was 24 to 36 mm (mean = 30 mm) and 0.27 to 1.15 g (mean = 0.62 g), respectively. The mean loading of fish in the dilution water control was 0.017 g/L/day. Records for any mortalities or symptoms of toxicity in the fish were made at 24, 48, 72 and 96 hours during the test.

Findings:

Validity criteria:

Validity criteria (according to OECD 203, adopted 17.07.1992)	**Obtained in this study**
Mortality in the controls (criterion is < 10%)	0%
Dissolved oxygen concentration in the control and test vessels (criterion is ≥ 60%)	> 60%

Temperature was 21.7 to 21.9 °C, *i.e.*, in the recommended range of 21 to 25 °C, and fish had a mean length of 30 mm, within the recommended length of 20 ± 10 mm.

Analytical findings:
Mean measured concentration was 108% of the nominal exposure concentration.
The pH values in the test ranged from 6.80 to 7.76, dissolved oxygen concentrations ranged from 8.8 to 9.0 mg/L, total hardness of the dilution water was 44.7 to 46.0 mg/L, conductivity of the water was 214 to 221 µS/cm and temperature ranged from 21.7 to 21.9 °C.

Conclusion
The acute toxicity study to *Lepomis macrochirus* has been investigated and gave the 96-hour NOEC of ≥ 120 mg/L and because no mortality was observed the LC_{100} was > 120 mg/L.

Report:	KCA 8.2.1/05 [redacted]; 2013; M-449083-01-1
Title:	Acute toxicity of fosetyl-aluminium (tech.) to fish (*Cyprinus carpio*) under static conditions (limit test)
Report No.:	EBFYL025
Document No.:	M-449083-01-1
Guideline(s):	U.S.-EPA-FIFRA § 72-1; SEP-EPA-540/9-85-006 (1982/1985) OCSPP 850.1075 (Public Draft, 1996) EC Council Regulation No 440/2008, Method C.1 (2008) OECD No. 203 (rev.1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of the limit test at 100 mg a.s./L was to demonstrate that fish (*Cyprinus carpio*) were not affected by the test item at this test level.

Material and Methods:
Test item: fosetyl-aluminium (tech.), analyzed content of active substance: fosetyl-aluminium: 98.1%; specified by batch code: AE F053616-01-11, Origin batch no.: 08001, tox no.: 09393-00.
Test organism: Common carp (*Cyprinus carpio*), mean body length 4.2 cm, mean body weight 1.2 g. Lot F 10 / 12 was delivered on July 18, 2012. The biomass loading for this test was 0.45 g fish tissue/L test medium. Thirty fish (fifteen fish per test vessel I and II) were exposed in a limit test for 96 h under static test conditions to a nominal concentration of 100 mg a.s./L against a water control with further 30 fish.
During the test, fish were examined after four hours and then daily for mortalities and signs of poisoning.
Within the study the pH-value, the oxygen saturation level and the temperature were measured with commercial measurement devices, daily.

Results:

Validity criteria:
Test conditions met all validity criteria, given by the mentioned guidelines: there was less than 5% mortality within the 48-hour settling-in period and ≤ 10% mortality in the control(s). Dissolved oxygen saturation was greater or equal to 60% throughout the test, and pH variations were ≤ 1.0 units.

Analytical results:
Dissolved oxygen concentrations ranged from 61 to 101% oxygen saturation, the pH values ranged from 6.5 to 7.6 and the water temperature ranged from 21.5 to 23.3°C in all aquaria over the whole testing period.
The analytical determination of fosetyl-Al (in water by HPLC – MS / MS) revealed a recovery of 87 to 92% of nominal over the whole testing period of 96 hours at the limit test concentration of 100 mg a.s./L. Therefore the results of this study are given based on the nominal concentrations.

Biological results:
There were neither any sub-lethal effects nor any mortality in the control group.
Cumulative mortality was observed as follows [with a total number of 30 (15 A + 15 B):

Exposure time	**4 h**		**24 h**		**48 h**		**72 h**		**96 h**	
Test level [mg a.s. / L]	**no. of dead**	**% dead**	**no. of dead**	**% dead**	**no. of dead**	**% dead**	**no. of dead**	**% dead**	**no. of dead**	**% dead**
Control I	0	0	0	0	0	0	0	0	**0**	**0**
Control II	0	0	0	0	0	0	0	0	**0**	**0**
100 I	0	0	0	0	0	0	0	0	**0**	**0**
100 II	0	0	0	0	0	0	0	0	**0**	**0**

Conclusion:

Test substance:	fosetyl-Al (tech.)
Test object:	Common carp (*Cyprinus carpio*)
Exposure:	96 hours, static design
LC_{50} 96 h (95% C.I.):	**> 100 mg a.s./L**
LOEC: lowest concentration with an effect	100 mg a.s./L
NOEC: highest concentration without toxic effects	< 100 mg a.s./L
NOLEC: highest concentration causing no mortality	100 mg a.s./L
100 % mortality:	> 100 mg a.s./L

The limit test showed that, at 100 mg a.s./L, fosetyl-Al (tech.) did not cause any mortality to Common carp *(Cyprinus carpio).* The 96h-LC_{50} is greater than 100 mg a.s./L.
Ten fish at test level (five fish each aquarium) showed the following symptoms after 96 hours: fish remained for unusually long periods at the water surface, showed labored respiration.
The 96h NOEC is < 100 mg a.s./L.

Report:	KCA 8.2.1/06 [redacted]; 2008; M-310496-01-1
Title:	Acute toxicity of phosphorous acid to fish (*Oncorhynchus mykiss*) under static conditions (limit test)
Report No.:	EBFYL007
Document No.:	M-310496-01-1
Guideline(s):	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev.1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

A limit test under static conditions at 400 mg/L was performed in order to show that fish (Rainbow trout) were not affected by phosphorous acid (H_3PO_3) at this test level.

Material and methods:

Test item: Phosphorous acid (H_3PO_3), applied as sodium-phosphite-dibasic-pentahydrate, analyzed purity: 102.3% (used as 100% pure, batch no. LOT 70090)

The toxicity of the fosetyl-Al metabolite phosphonic acid to rainbow trout, *Oncorhynchus mykiss*, (mean body length 4.6 cm, mean body weight 0.9 g) was determined under static test conditions over an exposure period of 96 h. The biomass loading during testing was 0.68 g fish tissue/L test medium.

Thirty fish were exposed to a water control and nominal test concentrations equal to 400 mg test substance/L (limit test). Dissolved oxygen, temperature and pH were measured at test initiation and after each test media renewal at 24, 48 and 72 hours. Recoveries of phosphonic acid were measured in all test levels on day 0, day 2 and day 4 of the exposure period to confirm nominal concentrations. Mortality and physical or behavioural alterations were recorded at 4, 24, 48, 72 and 96 hours after the start of exposure.

Findings:

The test conditions met all validity criteria, given by the mentioned guidelines.

Dissolved oxygen concentrations ranged from 87 to 100% oxygen saturation, the pH values ranged from 6.8 to 8.6 and the water temperature ranged from 11.8 to 13.4°C in all aquaria over the whole testing period.

Based on analytical determination of phosphonic acid mean measured values of 97% of nominal were found over the whole testing period of 96 hours. Therefore all results are given as nominal values.

No mortality or sublethal toxicity was observed in the control group as well as in the test group.

Conclusions:

Under the condition of the test, neither any mortality nor any sub-lethal effects occurred. The 96h-LC_{50} for fish is clearly above 400 mg phosphonic acid (H_3PO_3)/L.

CA 8.2.2 Long-term and chronic toxicity to fish

For more information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

To fulfill the data requirements of Commision Regulation (EU) No 283/2013 setting out the new data requirements for active substances, an Early Life Stage (ELS) study with fosetyl-aluminium was conducted with *Pimephales promelas* and is summarized in Section CA 8.2.2.1 (KCA 8.2.2.1/01, [redacted]; 2015; M-531353-01-1). This study resulted in a lower NOEC, which will be considered in the risk assessment.

CA 8.2.2.1 Fish early life stage toxicity test

Report:	KCA 8.2.2.1/01 [redacted]; 2015; M-531353-01-1
Title:	Early-life stage toxicity of fosetyl-Al (techn.) to fish (Pimephales promelas)
Report No.:	EBFYN029
Document No.:	M-531353-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation 1107/2009 (Europe) US EPA OCSPP 850.1400
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The objective of the study was to determine the chronic toxicity of fosetyl-aluminium (fosetyl-Al) to the early life-stages of fathead minnow (*Pimephales promelas*) under flow-through conditions.

Materials and methods:
Test item: Foseytl-Al (tech.), purity 98.0% w/w, specified by origin batch no.: 201407089, specification no.: 102000016699, Batch-Code: AE F053616-01-26.
Fathead minnow (*Pimephales promelas*), freshly fertilized eggs (< 24 hours old) were used at the start of the exposure period.
Early-life stages of fathead minnow (eggs, larvae/fry) were exposed to five test concentrations and a control under flow-through conditions with four replicates per treatment group over 32 days (28 days post-hatch). The biological parameters measured in this study were egg hatchability, survival and larval growth and morphological and behavioural effects.
The definitive study was conducted at the nominal test concentrations of 0.213, 0.470, 1.03, 2.27 and 5.00 mg a.s./L.
Water temperature was measured continuously in one control replicate aquaria. Over the entire test duration, temperatures ranged between 24.3 and 26.1 °C. Mean dissolved oxygen (DO) saturation ranged from 98 to 101% and the mean pH values varied between 7.0 and 7.3 in all aquaria over the whole test period. Measured light intensity ranged within 590 to 1075 lux with a photoperiod of 16 hours light and 8 hours dark.

Findings:

Analytical Findings:
The overall mean measured concentrations of fosetyl-Al during the test were 0.208, 0.493, 0.966, 2.39 and 5.41 mg a.s./L. The overall mean measured values correspond to recoveries of 94 to 108% of nominal for all test levels. The results of this study were therefore based on nominal concentrations of fosetyl-Al.

Validity:
The test fulfilled the validity criteria of the underlying guideline.

- The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test: The dissolved oxygen concentration ranged between 98 to 101%. Therefore the test fulfilled this validity criterion.
- The water temperature should not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test, and should be within 25 ± 1.5 °C. The water temperature ranged between 24.3 to 26.1 °C and did not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test. Therefore the test fulfilled this validity criterion.
- The compulsory analytical measurement of the test concentrations was performed.
- Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 respectively 75%: Overall survival of fertilised eggs was 92.5% and post-hatch success was 90.2%. Therefore the test fulfilled this validity criterion.

Toxicity to fish:
Based on morphological and behavioural observations and the statistical analysis of hatching success, fry survival and fry growth (expressed as wet weight, dry weight and total length), the test revealed the following NOEC, LOEC and EC_{10} (based on nominal concentrations of fosetyl-Al):

Test Substance	Fosetyl-Al		
Test Object	Fathead minnow *(Pimephales promelas)*		
Exposure	32 Day, flow-through (ELS)		
Results	**NOEC [mg a.s./L]**	**LOEC [mg a.s./L]**	**EC_{10} (95%-CL) [mg a.s./L]**
Hatching success (day 4)*	0.213	0.47	0.31 (0.05 – 0.56)
Larval Survival (day 32)	1.03	2.27	0.61 (0.48 – 0.73)
Growth (Length)**	0.47	1.03	1.18 (0.85 – 2.26)
Growth (Wet Weight)**	0.47	1.03	0.55 (0.01 – 0.75)
Growth (Dry Weight)**	0.47	1.03	0.42 (0.03 – 0.62)
Morphological & Behavioral Effects***	0.47	1.03	not applicable

*: Hatching started on day 3 and lastet until day 11. At concentrations > 0.213 mg a.s./L a statistically significant delay in hatching was observed. Development of embryos was similar at all test levels, but at higher concentrations a reduction in hatching success was statistically significantly. On day 11, the remaining unhatched embryos at 1.03 replicate B, 2.27 and 5.00 mg a.s./L were observed as being dead. Possibly the test item at these concentrations impacted the structure or stability of the chorion and consequently hampered the hatch of the larvae.

**: In the two highest test concentrations (2.27 and 5.00 mg a.s./L) only a low number of fish was available at the end of the test, due to reduced hatching. Since the growth of fish is density dependend, it was decided to exclude the respective concentrations from statistical analysis.

***: The results of morphological and behavioral effects are based on expert judgment. This type of data is not suitable for a statistical analysis.

Observations:
In the control and up to the test concentration of 0.470 mg a.s./L abnormal behaviour and abnormal appearance were observed only in very low numbers. These observations did not show any dose dependence and were not related to the test item, but mirror known background occurrence of this type of observations. Starting at 1.03 mg a.s./L up to the highest test concentration the number of observed abnormal behaviour and appearance increased and followed a dose response pattern.

Conclusions:
The overall chronic NOEC observed in this study is 0.213 mg a.s./L and the respective overall chronic LOEC is 0.470 mg a.s./L (based on hatching success).
Thus, considering that fosetyl-Al is rapidly degradable, fosetyl-Al may be classified according to the criteria of Regulation 1272/2008 as "Hazardous to the aquatic environment – Chronic Category 3" and "H412: Harmful to aquatic life with long lasting effects".

CA 8.2.2.2 Fish full life cycle test

Please refer to Section CA 8.2.2. Based on the triggers stated in the EU-directive and the Aquatic Guidance Document, a fish full life cycle (FFLC) study is not required.
The acute toxicity of fosetyl-aluminium (fosetyl-Al) for fish is >0.1 mg/L, and the chronic toxicity for fish is also low (see Section CA 8.2.2.1).
No bioconcentration factor in fish is triggered since log Pow values are <3 for fosetyl-Al and its metabolite phosphonic acid.

Report:	KCA 8.2.2.2/01 [redacted]; 1997; M-184572-01-1
Title:	Fish, juvenile growth test - 28 days Fosetyl-Al SPL Project number: 282/492
Report No.:	R011793
Document No.:	M-184572-01-1
Guideline(s):	OECD: Draft Guideline, (1994); Equivalent to US EPA OPPTS Guideline No. 850.1400
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The study was performed to assess the effect of fosetyl-Al on growth of juvenile rainbow trout (*Oncorhynchus mykiss*) over 28 days in a water renewal test system.

Materials and Methods:
Test item: Fosetyl-Al (tech.), analysed content of active substance: fosetyl-Al: 970 g/kg, specified by batch no.: OP9607181.
Test was carried out with juvenile rainbow trout (*Oncorhynchus mykiss*). Pre-exposure measurements showed the fish had a mean standard length of 6.1 cm (sd = 0.3) and a mean weight of 3.53 g (sd = 0.49). In the study 40 L glass exposure vessels were used for each replicate. Based on the mean weight value, this gave a loading rate at initiation of the study of 1.4 g fish tissue /L. Media used in the study was dechlorinated laboratory tap water with a total hardness of 134 mg/L as $CaCO_3$ at initiation and 105 mg/L as $CaCO_3$ at termination of the study. Groups of 16 juvenile rainbow trout in a single replicate were exposed to nominal concentrations of fosetyl-Al of 0 (control), 10, 18, 32, 56 and 100 mg/L for a period of 28 days. Test solutions were aerated and renewed every 24 hours with a photoperiod of 16 hours light and 8 hours darkness. Fish were fed daily at a rate of 4% body weight and the length and weight of each fish was determined at 0, 14 and 28 days. Mortalities and adverse reactions to exposure were recorded daily. Water temperature, pH and dissolved oxygen concentrations were recorded daily throughout the study. Test concentrations in solutions were verified by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using aluminum as marker. Water samples were taken from the control and all surviving test groups on days 0, 2, 6, 9, 13, 16, 20, 23, 27 (fresh media) and on days 1, 3, 7, 10, 14, 17, 21, 24 and 28 (old media).

Findings:

Analytical Findings:

Analytical verification of test concentrations showed actual test levels to be near nominal (64 to 108% recovery) over the 28 days study period. Based on the majority of sampling occasions, the measured concentrations were observed to be near nominal; it was thus considered justifiable to base the results in terms of nominal test concentrations only.

Temperature was maintained at 14 ± 1 °C. Mean values of pH and concentration of dissolved oxygen were 7.5 and 9.6 mg O_2/L, respectively. There were no treatment-related differences for oxygen concentration. Concentration-dependent differences in pH were observed in the freshly prepared test media throughout the study. Nevertheless, values obtained were within acceptable limits.

Validity:

The test fulfilled the validity criteria of the guideline.

- Mortality in the controls must not exceed 10% at the end of the test:
  No mortality was observed in the control treatment at the end of the study
- Mean weight of fish in the controls must have increased by at least 50% of their mean initial weight over 28 days:
  Mean weight of fish in the control increased by a factor of 2.5.
- The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test:
  The dissolved oxygen concentration was 9.6 mg O_2/L at the temperature of 14 ± 1 °C, thus > 60%.
- The water temperature must not differ by more than ± 1 °C between test chambers at any one time during the test and should be maintained within a range of 2 °C within the temperature range of 12.5 to 16.0 °C specified for *Oncorhynchus mykiss*:
  Temperature was 14 ± 1 °C, *i.e.*, in the recommended range for *O. mykiss*, and differed not more than ± 1 °C between test chambers at any one time during the test.

Biological findings:

Following 28 days of exposure, no mortality occurred in both exposed and control fish. None of the fish showed any behavioral effects. Inhibition of growth compared to control values did not exceed 13% (over the period 0 to 14 days) or 9% (over the period 0 to 28 days) at any of the test levels. Fish weight gain was comparable at all test levels.

Mean lengths and weights of rainbow trout at day 0, day 14 and day 28 of the study

Nominal concentration (mg/L)	Day 0				Day 14				Day 28			
	Length (cm)		Weight (g)		Length (cm)		Weight (g)		Length (cm)		Weight (g)	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Control	6.2	0.3	3.47	0.46	7.0	0.3	5.66	0.57	7.9	0.4	8.69	1.08
10	6.1	0.3	3.48	0.52	7.0	0.4	5.66	0.95	7.9	0.5	8.45	1.03
18	6.1	0.3	3.51	0.44	6.9	0.3	5.37	0.56	7.8	0.4	8.11	1.04
32	6.1	0.3	3.44	0.41	6.9	0.3	5.31	0.66	7.9	0.4	8.39	1.19
56	6.1	0.3	3.62	0.61	7.0	0.4	5.74	0.86	7.9	0.4	8.52	1.39
100	6.1	0.3	3.67	0.50	7.0	0.4	5.76	0.82	7.8	0.3	8.66	1.14

Conclusions:

The 28 day "No Observed Effect Concentration" (NOEC) is considered to be 100 mg a.s./L (based on nominal concentrations) on the basis that, at this test concentration, there were no mortalities, no behavioural effects, and no inhibition of growth observed. Furthermore, all validity criteria of the current guideline were fulfilled and the study can therefore be considered as valid.

❑ **Comments (RMS):** acceptable

CA 8.2.2.3 Bioconcentration in fish

Please refer to Section CA 8.2.2. A bioconcentration study in fish is not triggered, since $logP_{ow}$ values are < 3 for fosetyl-aluminium and its metabolite phosphonic acid.

CA 8.2.3 Endocrine disrupting properties

Fish
Population relevant effects of fosetyl-aluminium (fosetyl-Al) on fish were studied in a (28 d) juvenile growth test with rainbow trout and in an early life-stage test (ELS) with fathead minnow (see Section CA 8.2.2.1). In the 28-d study. no effects on growth or survival were seen at 100 mg/L. Based on the hatching success and growth parameters, a NOEC of 0.213 mg/L was found in the ELS.
Although they were not designed to provide comprehensive information on all endocrine endpoints, the available studies in fish showed no indication of endocrine-related effects. As there is also no indication of an endocrine disruption potential in mammals and birds, no further testing is indicated to evaluate the endocrine disrupter potential of fosetyl-Al to fish.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

For more information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The studies from which the endpoint will be used for risk assessment are summarised below.

One additional study on acute toxicity to *Daphnia magna* was performed, which was not submitted for Annex I inclusion of fosetyl under Directive 91/414/EEC and is submitted within this Supplementary Dossier for the approval renewal of fosetyl. This study is summarized below.

Report: KCA 8.2.4.1/01 [redacted] 1996; M-170914-01-1
Title: Fosetyl-Al: Acute toxicity to *Daphnia magna*
Report No.: R005424
Document No.: M-170914-01-1
Guideline(s): EU (=EEC): 92/69/EEC, C2; OECD 202, (1984); Equivalent to US EPA OPPTS Guideline No. 850.1010
Guideline deviation(s): not specified
GLP/GEP: yes

Objective:
The study was performed to assess the acute toxicity of fosetyl-Al to *Daphnia magna.*

Materials and methods:
Test item: Fosetyl-Al (tech.), analyzed content of active substance: fosetyl-Al: 959 g/kg, specified by batch no.: 955008.
Daphnia magna (1st instars < 24 h old, 10 x 4 animals per concentration) were exposed in a static test system for 48 hours to the nominal concentration of 100 mg a.s./L without feeding.
The control group was maintained under identical conditions but not exposed to the test material.
The test solutions were not renewed during the exposure period. Any immobilisation or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure.
The concentration of Fosetyl-Al in the test preparations at 0 and 48 hours was determined.
Temperature was maintained at 21 °C with a photoperiod of 16 hours light and 8 hours darkness.

Dates of experimental work: January 15, 1996 to January 17, 1996
Findings:

Analytical findings:
Analysis of the test preparations at 0 hours showed the measured concentrations to be near nominal. At 48 hours analysis of the unstirred test media showed a marked decline in measured test concentration. Analysis of the stirred test media at 48 hours showed the measured concentrations to be near nominal.

Biological findings:
There was no immobilisation in 40 daphnids exposed to a test concentration of 100 mg/L for a period of 48 hours.
There were no adverse reactions to exposure.
Toxicity of fosetyl-Al to *Daphnia magna*:

Test Concentration mg a.s./L Nominal	Exposed daphnids	Immobilised daphnids			
		24 h		48 h	
		n	% ± SD	n	% ± SD
Control	40	0	0	0	0
100	40	0	0	0	0

Conclusions:
There was no immobilisation in 40 daphnids exposed to a test concentration of 100 mg/L for a period of 48 hours. Inspection of the immobilisation data gave the following results:

Time (h)	EC_{50} mg a.s./L	95% CI mg a.s./L
24	> 100	n.d.
48	> 100	n.d.

n.d.: not determined

- **Comments (RMS):** acceptable

The summary presented above from the original DAR was already presented with further results and details for the submission of the fosetyl EU AR Supplementary Dossier.

Further study information supplementing the original DAR summary

Materials and methods:
Test was conducted with six replicate test vessels with duplicate control vessels.
Water temperature was recorded daily throughout the study. Dissolved oxygen concentrations and pH were recorded at the start and termination of the study. Test material concentrations were monitored by the determination of the aluminium concentration by inductivity coupled plasma emission spectrometry (ICP-AES). Water samples were taken from the control and the 100 mg/L test group at 0 and 48 hours.

Findings:

Validity criteria:

Validity criteria (according to OECD 202, adopted 13.04.2004)	Obtained in this study
Mortality in the controls (criterion is < 10%)	0 %
Dissolved oxygen concentration in the control and test vessels (criterion is ≥ 3 mg/L)	≥ 7.9 mg/L

<u>Analytical findings:</u>
Analysis of the test preparations at 0 hours showed the measured concentrations to range between 88 and 90% of nominal (mean 89%). At 48 hours analysis of the unstirred test media showed a marked decline in measured test concentration (5% of nominal). Analysis of the stirred test media at 48 hours showed the measured concentration to be near nominal (85 to 88%) In order to give at "worst case" analysis of the data, it was considered justifiable to base the results on the 48-hour mean measured concentration.

Nominal concentration (mg/L)	**0 hours**			**24 hours**	**48 hours**		
	pH	**mg O_2/L**	**T °C**	**T °C**	**pH**	**mg O_2/L**	**T °C**
Control[1]	7.7 - 7.7	8.5 - 8.5	21.0 - 21.0	21.0 - 21.0	7.7 - 7.7	7.9 - 8.0	21.0 - 21.0
100[2]	5.9 - 6.0	8.5 -8.5	21.0 - 21.0	21.0 - 21.0	6.2 - 6.3	8.1 - 8.2	21.0 - 21.0

[1] conducted with 2 replicates
[2] conducted with 6 replicates whereas 2 contained no daphnids

Conclusion
The acute toxicity study of fosetyl-Al to *Daphnia magna* has been investigated and gave the 48-hour EC_{50} of ≥ 100 mg/L. Correspondingly the NOEC was ≥ 100 mg/L.

Report:	KCA 8.2.4.1/02; [redacted]; [redacted]; 1994; M-179068-01-1
Title:	Potassium salts of phosphorous acid: Acute toxicity to the water flea, *Daphnia magna*, under static conditions.
Report No.:	R009322
Document No.:	M-179068-01-1
Guideline(s):	USEPA (=EPA): FIFRA No.154-9
Guideline deviation(s):	not specified
GLP/GEP:	yes

Methods:
Ten water fleas were impartially assigned to each test container within 30 minutes after the addition of the test substance (purity: 400 g phosphorous acid/L), at a nominal test concentration of 100 mg/L. The control (dilution water) and test substance treatment were triplicated.

Results:
The mean measured concentration of potassium salts of phosphorous acid was 100 mg/L which was 100% of nominal. Mortality (or immobilization) of daphnids was 0% in both treated and control groups.

Conclusions:
EC50 - 48 h > 100 mg/L (nominal concentrations) (> 29.7 mg H_3PO_3/L)
NOEC - 48 h = 100 mg/L (nominal concentrations) (= 29.7 mg H_3PO_3/L)

❑ **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Objective:
The study was performed to assess the acute toxicity of potassium salts of phosphorus acid to *Daphnia magna*.

Materials and methods:

Test item: potassium salt of phosphorus acid (Batch No.: #2244-3, lot No.: CSI#64-F5B, purity = 410 g/L).

The test was conducted with < 24 hours old neonates in moderately hard freshwater with a hardness of 66 mg/L as calcium carbonate ($CaCO_3$) and a conductivity of 382 µS/cm. The bioassay was conducted over a duration of 48 hours and survival of water fleas was monitored daily. Any dead or immobilized water flea was removed. Daphnids were not fed during the test.

Water temperature was recorded daily throughout the study. Dissolved oxygen concentrations and pH were recorded at the start and termination of the study. Test item concentrations were verified at test initiation and termination by titrimetric analysis following method LTM-017.

Findings:

Validity criteria:

Validity criteria (according to OECD 202, adopted 13.04.2004)	**Obtained in this study**
Mortality in the controls (criterion is < 10%)	0 %
Dissolved oxygen concentration in the control and test vessels (criterion is ≥ 3 mg/L)	≥ 8.4 mg/L

Analytical findings:

Nominal concentration (mg/L)	**0 hours**			**24 hours**	**48 hours**		
	pH	**mg O_2/L**	**T °C**	**T °C**	**pH**	**mg O_2/L**	**T °C**
Control	7.3	9.0	20.5	20.2	7.4 - 7.4	8.4 – 8.6	20.9
100	6.4	8.9	-	-	6.8 - 6.8	8.4 – 8.5	-

Conclusion

The acute toxicity study of potassium salts of phosphorus acid resulted in zero percent mortality in *D. magna* at 100 mg/L after 48 hours, thus delivering a 48-hour EC_{50} ≥ 100 mg/L. Accordingly, the NOEC was ≥ 100 mg/L.

Report:	KCA 8.2.4.1/03 [redacted]; 2008; M-310318-01-1
Title:	Acute toxicity of phosphorous acid to the waterflea *Daphnia magna* in a static laboratory test system
Report No.:	EBFYL006
Document No.:	M-310318-01-1
Guideline(s):	OECD guideline 202 (2004); EEC Directive 92/69/EEC, part C.2 (1992); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000).
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The objective of the reported study was to verify the absence of treatment-related effects on mobility of *Daphnia magna* over 48 hours under static exposure conditions, when exposed to a limit concentration of 400 mg phosphorous acid/L.

Material and methods:

Test item: Phosphorous acid (H_3PO_3), applied as sodium-phosphite-dibasic-pentahydrate, analyzed purity: 102.3% (used as 100% pure, batch no. LOT 70090)

The toxicity of the fosetyl-aluminium metabolite phosphonic acid to *Daphnia magna* was determined under static test conditions. Five daphnids per replicate, ten replicates per concentration, were exposed for 48 h to a water control and nominal test concentrations of 400 mg test substance/L. The test solutions were sampled and analyzed at the beginning and the end of the test period.

Dissolved oxygen and pH were measured at test initiation and termination. Temperature was recorded throughout the study. Immobilization and adverse reactions were recorded at 24 and 48 hours after the start of exposure.

Findings:
Mean measured values of 106 and 117% of nominal of phosphonic acid were found at beginning and at end of the testing period of 48 hours. Therefore, all results are given as nominal values.

No mortality or sublethal toxicity was observed in the control groups as well as in the test groups.

Conclusions:
Under the condition of the test, neither any mortality nor any sublethal effects occurred. The 48 h-EC_{50} for *Daphnia* is clearly above 400 mg phosphonic acid (H_3PO_3)/L.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

No acute studies on an additional aquatic invertebrate species are required since fosetyl-aluminium is not an insecticide and does not show an insecticidal mode of action.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The study from which the endpoint will be used for risk assessment is summarised below.

Report:	KCA 8.2.5.1/01 [redacted]; 1996; M-189214-01-1
Title:	Fosetyl-Al: *Daphnia magna* reproduction test
Report No.:	R014229
Document No.:	M-189214-01-1
Guideline(s):	OECD: 202, (1984); Equivalent to US EPA OPPTS Guideline No. 850.1300
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The aim of the study was to assess the effect of fosetyl-Al on the reproduction of *Daphnia magna* over a 21 day period.

Materials and methods:
Test item: Fosetyl-Al (tech.), analyzed content of active substance: fosetyl-Al: 959 g/kg, specified by batch no.: 9550083.
Based on the results of an acute toxicity study, *Daphnia magna* were exposed (4 replicates of 10 daphnids per group) to an aqueous dispersion of the test material over a range of test concentrations of 1.0, 3.2, 10, 32 and 100 mg/L for a period of 21 days. The test solutions were renewed 3 times per week. The numbers of alive and dead adult *Daphnia* were determined daily. The numbers of young *Daphnia* (alive and dead) were determined at each test media renewal. The *Daphnia* were fed daily with a mixed algal suspension.
Temperature was maintained at 21°C with a photoperiod of 16 hours light and 8 hours darkness for a period of 21 days.

Dates of experimental work: February 12, 1996 to March 4, 1996

Findings:

Validity criteria:

Validity Criteria	Recommended	Obtained
Control mortality	≤ 20%	0%
Dissolved oxygen	≥ 60%	88%
pH (controls)	Deviation ≤ 0.3	0.1
First young (control group)	Produced within 9 days	7 days
Cumulative young per female	≥ 20 after 14 days	22
Cumulative young per female (control group)	≥ 40 after 21 days	49
Number of broods per control group	≥ 3	

Analytical findings:
Analysis of the freshly prepared test media on day 0 showed the measured concentrations to be near nominal. Analysis of the expired test media showed a marked decline in measured concentrations with concentrations ranging from 0 to 96% of nominal. These low and variable results are considered to be due to analytical variation given that the theoretical concentration of aluminium at the lowest test concentrations is near to the limit of detection of the analytical method, adsorption to the glassware and/or algal cells given as feed to the daphnids, and problems associated with sampling from heterogeneous dispersion. It was therefore considered justifiable to give the results based on the mean measured test concentrations.

Biological findings

Lethal Effects on the Parental Generation:
Mortality (immobilisation) occurred predominantly within the first 96 hours of exposure at the highest nominal test concentration of 100 mg/L resulting in 100% mortality by day 5. No mortalities occurred at the test concentrations of 1.0, 3.2, 10 and 32 mg/L throughout the duration of the study.
There was a significant effect on size and colouration of the daphnids as a result of exposure to fosetyl-Al in that the surviving daphnids at the test concentration of 100 mg/L were markedly smaller in size and paler in colour than the control animals on day 4 until 100% immobilisation was observed on day 5.
The daphnids at the test concentration of 1.0, 3.2, 10 and 32 mg/L were observed to be the same size and colour as the control animals.

Sublethal Effects on the Parental Generation:
After both 14 and 21 days, there were no statistically significant differences between the controls and the 1.0, 3.2, 10 and 32 mg/L test groups in terms of the number of young produced per adult. The 100 mg/L test group produced no young on days 14 and 21 due to 100% mortalities being observed prior to these time points.

Effects on the Filial Generation:
Information on the effects of fosetyl-Al on the F1 generation is limited, since, by study design, the young are removed soon after liberation from the brood pouch. However, an assessment made at each media renewal showed the "filial" daphnids produced by the 1.0, 3.2, 10 and 32 mg/L test groups were in the same general condition as the young produced by the controls over the duration of the study.
Numbers of unhatched eggs and dead young were low in all control and treatment groups surviving to maturation.

Effects of fosetyl-Al to *Daphnia magna*:

Nominal concentration (mg/L)	Mean measured test concentration (mg/L)	% survival of P1	No. of live young		No. of dead young		No. of unhatched eggs	
			Total	Per female (cumulative)	Total	Per female (cumulative)	Total	Per female (cumulative)
Control	Control	100	1962	49	3	<1	3	<1
1.0	0.47	100	1826	46	2	<1	7	<1
3.2	1.97	100	1884	47	3	<1	6	<1
10.0	4.54	100	1872	47	4	<1	3	<1
32.0	17.0	100	1807	45	2	<1	3	<1
100.0	88.6	0	0	0	0	0	0	0

Conclusions:
The "No Observed Effect Concentration" (immobilisation and reproduction) is considered to be 17 mg/L based on mean measured test concentrations on the basis that at this test concentration there were no mortalities (immobilisation) observed in the parental generation (P_1) and that there were no significant differences ($P \geq 0.05$) between the control and the 17 mg/L test group in terms of numbers of young produced per adult on days 14 and 21.

- **Comments (RMS):** acceptable

The summary presented above from the original DAR was already presented with further results and details for the submission of the fosetyl EU AR Supplementary Dossier.

Further study information supplementing the original DAR summary:

Amendment
Validity criteria, according to OECD 211, adopted 02.10.2012.

Materials and methods:
Test was conducted in reconstituted water which was the same as that used to maintain the stock animals. The numbers of alive and dead adult *Daphnia* were determined daily and the number of young *Dapnia* (alive and dead) were determined at each test media renewal.
Temperature of the test solutions was recorded daily throughout the study. Dissolved oxygen concentration, pH and temperature were recorded before and after each test media renewal.
Test item concentrations were monitored by the determination of the aluminium concentration by inductivity coupled plasma emission spectrometry (ICP-AES).

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

Please refer to Section CA 8.2.5. Due to the low toxicity of fosetyl-aluminium observed in above tests, no further testing was deemed necessary.

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

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

CA 8.2.5.3 Development and emergence in *Chironomus* species

Please refer to Section CA 8.2.5. The three main criteria considered for deciding the need of conduct testing on invertebrates living in the sediment are persistence in soil, in aquatic environments, adsorption to sediment and aquatic invertebrates toxicity.
Fosetyl-aluminium (fosetyl-Al) is not persistent in soil or water, does not partition into the sediment and is practically non-toxic to representative invertebrate species (*Daphnia magna*).

As fosetyl-Al does not trigger the endpoint values for any of the three criteria, it is justified that there is no need to conduct this test with the parent compound.
However, a chronic study has been conducted with the main fosetyl-Al metabolite, phosphonic acid (see Section CA 8.2.5.4).

CA 8.2.5.4 Sediment dwelling organisms

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The study from which the endpoint will be used for risk assessment is summarised below.

Report:	KCA 8.2.5.4/01 [redacted]: 1999; M-171912-01-1
Title:	EXP10679A (Potassium salt of phosphorous acid): Toxicity to the sediment dwelling chironomid larvae (*Chironomus riparius*) - 28 days
Report No.:	R003571
Document No.:	M-171912-01-1
Guideline(s):	BBA: (1995); Equivalent to US EPA OPPTS Guideline No. 850.1790
Guideline deviation(s):	1. Biological observations were not performed on Day 17 of the test, the study protocol stated that daily observations would be performed during the emergence period of adult midges. 2. The volume of dilution water in each test vessel was 2 600 ml instead of 2 500 ml as stated in the protocol according to analytical measurements.
GLP/GEP:	**yes**

Objectives:
The purpose of this study was to estimate the toxicity of EXP10679A (potassium salts of phosphorous acid) hereafter referred to as EXP10679A on the sediment-dwelling life stage of *Chironomus riparius* in a static sediment-water system.

Materials and Methods:
Test item EXP10679A (potassium salts of phosphorous acid), analysed purity: 409 g/L phosphonic acid, specified by batch no.: OR960971.
A total of 500 organisms (25 per replicate, 4 replicates per test group) were exposed to 4 concentrations of EXP10679A and a dilution water-sediment control for an exposure period of 21 days. The definitive test was carried out using the nominal concentrations of EXP10679A of 42.1, 84.3, 168.5 and 337 mg/L. The corresponding nominal concentrations of phosphonic acid were as follows 12.5, 25.0, 50.1 and 100.2 mg/L.

The dissolved oxygen concentration remained above or equal to 4.0 mg/L., the water pH values ranged from 8.56 to 8.77 and the water temperature ranged from 20.7 to 21.9 °C.
The test solutions were sampled and analyzed for the presence of phosphorous acid one hour after test initiation, after 7 days and at test termination (Day 21).

Dates of work: November 26, 1998 to December 17, 1998

Findings:
One hour after test initiation, analytical verification of the nominal test concentrations of phosphorous acid in the overlying dilution water demonstrated the measured values to be close to the nominal concentrations (85 to 115% recovery).
Seven days after application of the test substance, the measured concentrations of phosphorous acid remained stable in the overlying water (88 to 104% recovery of the initial measured values).
At test termination (Day 21), percentage recoveries (compared to the initial measured values) of the three highest concentrations of phosphorous acid were 96, 117 and 88%, respectively. The lowest concentration of 12.5 mg/l of phosphorous acid slightly decreased with 64% recovery compared to the initial measured concentration.
The results of this test are reported in terms of nominal concentrations of EXP10979A expressed as milligrams per liter (mg/L).

The validation criteria for pH (6.0 to 9.0 units) and oxygen (>2.5 mg/L) were both met.

Biological results:
At least 84% emergence was observed in each replicate of the dilution water control group and the mean development rate for larvae in this group was 0.088 (range 0.085 to 0.090).
Therefore, the biological validation criteria in the control group were fulfilled i.e. emergence 70% and mean development rate within the range 0.05 - 0.1).
84 to 96% emergence was recorded from the test vessels exposed to the test substance and the mean development rate for larvae in these groups ranged from 0.087 to 0.089.
No physical or behavioral alterations were observed in any of the test groups compared to the control.
The emergence of adult midges from first instar larvae was not significantly reduced at any of the concentrations tested. No significant effect on the development rate of adult midges was observed at any of the concentrations tested.

Influence on emergence and development rate after 21 days (based on nominal initial concentrations of the test item in the overlying water):

Concentration nominal mg test item/L	Number of emerged midges	Emergence of inserted larvae Total (%)	Development rate (pooled sex) (1/d)
Control	90	90.0	0.088
42.1	87	87.0	0.088
84.3	91	91.0	0.087
168.5	91	91.0	0.088
337.0	89	89.0	0.087

Test conditions met all validity criteria given by the mentioned guideline.

Conclusions:
The No Observed Effect Concentration (NOEC) is estimated to be the highest test substance concentrations of 337.0 mg/L of EXP10679A corresponding to 100.2 mg/L of the active ingredient phosphorous acid.

- **Comments (RMS):** acceptable

The summary presented above from the original DAR was already presented with further results and details for the submission of the fosetyl EU AR Supplementary Dossier.

Further study information supplementing the original DAR summary:

Materials and methods:
The test was conducted with first instar larvae (2 to 3 days old) in 3 L glass beakers measuring 10 to 13 cm in diameter and with a height of approximately 27.5 cm. Dilution water consisted of reconstituted water (80% DSW, 20% LC-oligo) and was the same used in daphnid testing. Sediment used in the test was artificial sediment containing 10% sphagnum peat, 20% kaolin clay and 70% industrial sand. Before testing, each beaker was filled with artificial sediment to a height of 2 cm and left to stand for 24 hours. Overall, each test vessel contained 200 g of sediment and 2.6 L of dilution water (depth of approximately 20 cm).
During the test, larvae were fed at least 3 times per week at a rate of approx. 1 mg fish food per day per larvae, and food was reduced to 50% when 50% of midges had emerged. Lighting was provided with a photoperiod of 16 hours light : 8 hours darkness at an intensity of 1012 to 1145 lux.
During the period of emergence, a daily check of emerged midges was performed, and sex and number of adults emerging was recorded at each observation time. Concentration of test substance in the test solutions was carried out by a titrimetric method. The limit of quantification of this method was approx. 10 mg/L.

Findings:

Validity criteria:

Validity criteria (according to OECD 219, adopted 13.04.2004)	**Obtained in this study**
Emergence in the controls (criterion is ≥ 70%)	90 %
Dissolved oxygen concentration in the control and test vessel (criterion is ≥ 60%)	> 60 %
pH in the control and test vessel (criterion is between 6 to 9)	7.3 to 7.5
Water temperature should not differ by more than ± 1 °C	20.7 to 21.9
Period of *C. riparius* emergence to adults from control vessels (criterion is between 12 and 23 days)	11 to 14

Biological results:

Endpoint	**After 21 days**
NOEC [mg/L]	100.2
LOEC [mg/L]	>100.2

Conclusion
Under laboratory conditions, the 21-day no observed effect concentration (NOEC) is estimated to be the highest test substance concentrations of 337.0 mg/L of EXP10679A corresponding to 100.2 mg/L of phosphorous acid. The lowest observed effect (LOEC) was reported to be in excess of the highest test concentration of 337.0 mg/L of EXP10679A (corresponding to 100.2 mg/L of phosphorous acid).

Request from the RMS:
The chronic toxicity endpoint for *Chironomus riparius* (phosphonic acid) should also be expressed in mg a.s./kg sediment as phosphonic acid has a potential of accumulation in the sediment.

Response from BCS:
The chronic toxicity endpoint of phosphonic acid for *Chironomus riparius* is derived from the study by [redacted], 1999; M-171912-01-1, which provided a NOEC > 100.2 mg/L. In this study, phosphonic acid concentrations were measured only in the overlying water after 1 hour, 7 and 21 days. The analytical results (see Table 2 in the study report) show that the recovery of phosphonic acid was close to 100%, without decrease with time, for the three highest concentrations tested (*i.e.*, 25, 50.1 and 100.2 mg/L). This indicates that, over the experimental period, phosphonic acid remained in the water phase, and did not accumulate in the sediment. Results were therefore expressed with respect to the matrix where phosphonic acid was present (*i.e.*, the overlying water), so as mg/L.

CA 8.2.6 Effects on algal growth

For more information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The studies from which the endpoint will be used for risk assessment are summarised in the following sections.

CA 8.2.6.1 Effects on growth of green algae

Two additional studies on effect on the growth of green algae and a recalculation of an EC_{50} value were performed, which were not submitted for Annex I inclusion of fosetyl under Directive 91/414/EEC and are submitted within this Supplementary Dossier for the approval renewal of fosetyl. These studies are summarized below.

Report:	KCA 8.2.6.1/01 [redacted]; 1999, M-189220-01-1
Title:	Fosetyl-Al: Algal growth inhibition assay on *Scenedesmus subspicatus*
Report No.:	R014235
Document No.:	M-189220-01-1
Guideline(s):	EU (=EEC): 67/69/EEC, Part C, Method 3; OECD: 201; Equivalent to US EPA OPPTS Guideline No. 850.5400
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objectives:
The study was conducted to assess the inhibitory effect of fosetyl-aluminium (fosetyl-Al) on the growth of the unicellular green algae *Scenedesmus subspicatus*, expressed as NOEC and EC_X for growth rate and algal biomass (cells per volume).

Methods:
The following nominal concentrations of fosetyl-Al (purity: 978 g/kg) were used: 0.68,1.5, 3.3, 7.3, 16 and 35 mg/L.

Results:
Mean measured concentrations were 0.64, 1.4, 3.0, 7.1, 16 and 36 mg/L. Cell counts in the 36 mg/L group were considered inaccurate because the algal cells were clumping around the rim of the flask and in the media, which make the count very difficult in this group. For this reason the calculation of toxicity values were made for both cases, with and without the data for the 36 mg/L test level. Taking into account the inaccuracy of counts at 36 mg/L, it was considered that the toxicity values most reliable were those corresponding to data which excluded such test level.

Conclusions:

Taking into account the highest dose level:	
Yes	No
ErC50 - 72 h > 16 mg a.s./L (mean measured concentrations) EbC50 - 72 h = 6.1 mg a.s./L (C.I. 95% : 5.1 – 7.3 mg/L) NOEC - 72 h = 1.4 mg a.s./L	ErC50 - 72 h > 16 mg a.s./L (mean measured concentrations) EbC50 - 72 h = 5.9 mg a.s./L (C.I. 95% : 5.3 – 6.6 mg/L) NOEC - 72 h = 1.4 mg a.s./L

- **Comments (RMS):** acceptable. Calculations excluding the highest dose were considered valid.

Request from the RMS:
Further explanations are required to justify that the study of the effects of the preparation on *Scenedesmus subspicatus* ([redacted],; 1999; M-189220-01-1) are still reliable for the risk since the coefficient of variation is estimated to be 57% for the control.

Response from BCS:
The RMS is right: the study by [redacted],; 1999; M-189220-01-1 does not meet the validity criteria according to the OECD TG 201. BCS´s calculations resulted in a value of 57.2% for the mean coefficient of variation for section-by-section specific growth rates of controls.
However, a new, more recent study was made available [redacted] 2007; M-289324-01-1, which fulfills the validity criteria according to the OECD TG 201 and therefore delivers reliable endpoints that can be considered for the risk assessment of fosetyl-Al.

Report:	KCA 8.2.6.1/02[redacted]; 1999; M-171844-01-1
Title:	EXP10679A (Potassium salt of phosphorous acid): Toxicity to the green alga *Selenastrum capricornutum*
Report No.:	R005933
Document No.:	M-171844-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, C3; OECD: 201, (1984); OCSPP 850.5400
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objectives:
The aim of the study was to determine the influence of EXP10679A (potassium salts of phosphorous acid) on exponentially growing *Selenastrum capricornutum* expressed as NOEC, LOEC and EC_X for growth rate of algal biomass (cells per volume).

Materials and Methods:
Test item: EXP10679A (potassium salts of phosphorous acid), analysed purity: 409 g/L phosphorous acid, specified by batch no.: OP960971.
Selenastrum capricornutum were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 0.73, 1.6, 3.7, 7.7, 17, 37, 82 and 180 mg/L in comparison to a culture medium control. The test system consisted of three replicate vessels per test level and six replicate vessels per control. The initial cell number was 10,000 cells/mL.
Growth inhibition was calculated using algae biomass per volume. The surrogate for biomass was cell density (used as response parameter).
The pH values ranged from 7.7 to 9.6 in the controls and the incubation temperature ranged from 24.0 to 24.4 °C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7680 lux.

Findings:
Validity of the study:

Validity Criteria:	Obtained in this study:
Increase of biomass:	Biomass increased in the control by more than 16-fold within the evaluation period.

Analytical results:
The measured concentrations at the start of the test ranged from 82 to 108% of the nominal values. The measured concentrations in the blanks after 72 hours ranged from 100 to 124% of the nominal values. The overall mean measured concentrations determined ranged from 94 to 116% of the nominal values. On the basis of the analytical results the nominal concentrations were used for the calculation and reporting of all results.

Biological results:

Effect of EXP10679A on Freshwater Algae (*Selenastrum capricornutum*) in a 72 h growth inhibition test

Nominal concentration [mg test item/L]	Cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	Percentage of culture medium control [%]
Culture medium control	2 790 000	1.848	--
0.73	2 890 000	1.860	101
1.6	3 010 000	1.874	101
3.5	2 730 000	1.841	100
7.7	2 630 000	1.829	99
17	1 860 000	1.713*	93
37	483 000	1.264*	68
82	150 000	0.873*	47
180	106 000	0.759*	41

test initiation with 10,000 cells/mL
* Significant difference (P=0.05) from the culture medium control

Conclusions:
The (0 - 72h)-E_rC_{50} is 99 mg test item/L (29.4 mg phosphorous acid/L).

❑ **Comments (RMS):** acceptable

The summary presented above from the original DAR was already presented with further results and details for the submission of the fosetyl EU AR Supplementary Dossier.

Further study information supplementing the original DAR summary:

Materials and methods:
Test vessels were conical flasks of 250 mL nominal capacity. During test, cultures were shaken at 160 rpm. Six replicate cultures of the control and triplicate cultures of each concentration of the test substance were employed. The control consisted of culture medium. Cell density was obtained every 24 hours. Concentration of test substance in the test solutions was carried out by a titrimetric method. The limit of quantification of this method was approximately 10 mg/L.
The areas under the growth curve (biomass) and average growth rate of each replicate culture was calculated for day 0 to 3.

Findings:

Validity criteria:

Validity criteria (according to OECD 201, adopted 23.03.2006)	Obtained in this study
	control
Biomass increased in the control within the evaluation period (criterion is ≥ 16-fold).	279-fold
Mean coefficient of variation for section by section specific growth rates (days 0-1, 1-2, 2-3) in the controls (criterion is ≤ 35%)	11.1%
Coefficient of variation for average specific growth rates during the 0 to 72 hour test period in replicate control cultures (criterion is ≤ 10%).	2.97%

Biological results:

Endpoint	After 72 hours
E_bC_{50} [mg/L] (95% CI)	29 (15 – 75)
NOEC	7.7
LOEC	17
E_rC_{50} [mg/L] (95% CI)	99 (36 – 180)
NOEC	7.7
LOEC	17

Conclusion

Under laboratory conditions, EXP10679A (potassium salts of phosphorous acid) had an effect on the biomass and the growth rate of *Selenastrum capricornutun*. The 72 hour E_bC_{50} was calculated to be 29 mg/L (15 to 75 mg/L, 95% CI) and the 72 hour E_rC_{50} was calculated to be 99 mg/L (36 to > 180 mg/L, 95% CI) The NOEC (no observed effect concentration) and the LOEC (lowest observed effect concentration) was calculated to be 7.7 and 17 mg/L, respectively.

Report:	KCA 8.2.6.1/03 [redacted].; 1989; M-163526-01-1
Title:	The toxicity of fosetyl-Al to *Selenastrum capricornutum*.
Report No.:	R002887
Document No.:	M-163526-01-1
Guideline(s):	USEPA (=EPA): J, 123-2
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objectives:

The objective of the study was to determine the 7-day EC_{25} and EC_{50} values of fosetyl-aluminium (fosetyl-Al) for *Selenastrum capricornutum*. The no observed effect concentration (NOEC) was also determined.

Materials and Methods:

Test item: Fosetyl-Al technical, Batch #DA498, 98.5% (a.s.).

Selenastrum capricornutum was exposed over a 7-day period to six concentrations (1.0, 1.8, 3.2, 5.6 and 10 mg/L nominal) of fosetyl-Al technical and a control. Biomass was determined by cell counts on days 2, 3, 4 and 7.

Percent inhibition, relative to the control, was calculated for each concentration based upon the mean standing crop in cells/mL at 7 days. The EC_{25} and EC_{50} values were determined by weighted least squares nonlinear regression of the log of concentration against cell counts.

The test system consisted of three replicate vessels per test level and per control. The initial cell number was 3000 cells/mL.

The pH values were in the range of 7.5 ± 0.1 in the controls, and the incubation temperature was in the range of 24 ± 2.0°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 4306 ± 646 lux was given.

Samples were analyzed for the actual concentration of fosetyl-Al present in the test solutions on Day 0 and at the end of the test.

Dates of experimental work: December 12 1988 to December 19 1988

Results:

Analytical results:

The mean measured concentrations were 1.33, 2.28, 4.28, 9.01 and 12.50 mg/L. When calculating the mean, the unusually low value on Day 0 for the highest test concentration was omitted.

Biological results:

Effect of fosetyl-Al on freshwater algae (*Pseudokirchneriella subcapitata*) in a 7 d growth inhibition test

Mean measured concentration [mg a.s./L]	Cell number after 7 days (means) per mL	Inhibition of mean standing crop after 7 d [%]
Control	2 793 333	--
1.33	2 720 000	2.6
2.28	2 353 333	15.8
4.28	1 766 667	36.8
9.01	151 667	94.6
12.5	9 667	99.7

Conclusions:
The 7-day EC_{25} is 3.89 mg/L (95% confidence limits 3.53 to 4.29 mg/L) and the 7-day EC_{50} is 4.99 mg/L (95% confidence limits 4.63 to 5.36 mg/L).
The no observed effect concentration (NOEC), based upon the mean standing crop values on day 7, was determined by an analysis of variance and Dunnett's test to be 2.28 mg/L.

Request from the RMS:
It should be indicated if the study of the effects of fosetyl-Al to *Selenastrum capricornutum* ([redacted].; 1989; M-163526-01-1) respects the validity criteria at least on the period of 3 days (i.e. the number of cells after 24h should be reported). Could you, please, provide these data to allow the validation of the toxicity endpoints from this study? In addition, if the coefficient of variation is above 35% some justifications would be necessary to demonstrate the reliability of the results of this study.

Response from BCS:
The validity of the study on *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) by [redacted].; 1989; M-163526-01-1 has been checked. As noted by the RMS, cell density at 24 hours is missing. The criteria were therefore checked over a 72-hour period from day 2 to day 4. In these conditions, the study meets the validity criteria according to the OECD TG 201. In particular, the mean coefficient of variation for section-by-section specific growth rates of controls was 16.6%, and the coefficient of variation of average specific growth rates in replicate controls was 0.239%. Such low CV values in the control replicates indicate a small dispersion of the data. In addition, visual inspection of the growth curves shows that there was no inflexion within the 0-96 h period in the control. These warrant the robustness of the comparison between control and exposed algae.
In addition, the study by [redacted] benefited from a complete re-calculation, using the ToxRatPro Software ([redacted]; 2005; M-253825-01-1). The purpose of this re-calculation was to derive toxicological endpoints according to the requirements of the OECD TG 201. This re-calculation provided a 72-h E_rC_{50} of 9.54 mg/L, a LOE_rC of 4.28 mg/L, and a NOE_rC of 2.28 mg/L (see KCA 8.2.6.1/04).

Report:	KCA 8.2.6.1/04 [redacted]; 2005; M-253825-01-1
Title:	*Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*) - growth inhibition test with Fosetyl - Al
Report No.:	DOM 25005
Document No.:	M-253825-01-1
Guideline(s):	Originally reported under US-EPA FIFRA § 122-2 and 123-2 ; Recent recalculation is based on OECD 201 (June 1984) "Alga, Growth Inhibition Test" under consideration of the new draft revised proposal for updating OECD 201 (Oct. 22, 2004)
Guideline deviation(s):	none
GLP/GEP:	**no**

Objectives:

The aim of this non-GLP recalculation report was to fulfil the new OECD guideline 201 requirements, which ask for the EC_{50} (0-72h) for growth rate.

Materials and Methods:

Recalculation was done using the commercial program ToxRat Professional[1,2].

Results:

Cell numbers, average growth rates and % inhibition:

Mean measured concentration [mg a.s./L]	Cell number after 0 h per mL	Cell number after 72 h per mL	(0-72h)-average growth rate	Inhibition of average growth rate (0-72h) [%]
Control	3 000	208 000	1.413	--
1.33	3 000	221 333	1.433	-1.4
2.28	3 000	157 000	1.317	6.8
4.28	3 000	115 667	1.205	14.7
9.01	3 000	28 667	0.751	46.8
12.5	3 000	14 000	0.512	63.8

Conclusions:

Results based on OECD 201 are:

E_rC_{50} (0 – 72 h): 9.54 mg/L
LOE_rC (0 – 72 h): 4.28 mg/L
NOE_rC (0 – 72 h): 2.28 mg/L

1 Statistical Software "ToxRat Professional", version 2.09, produced by ToxRat®Solutions GmbH, 52477 Alsdorf, Germany (Feb 6, 2004)

2 ToxRat® Validation Document from ToxRat®Solutions GmbH, valid for ToxRat® Version 2.09 (released January 25, 2004)

Report:	KCA 8.2.6.1/05 [redacted]; 2007; M-289324-01-1
Title:	*Desmodesmus subspicatus* growth inhibition test with Fosetyl-Al
Report No.:	EBFYX019
Document No.:	M-289324-01-1
Guideline(s):	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:

The aim of the study was to determine the influence of the test item on exponentially growing *Desmodesmus subspicatus* expressed as NOEC, LOEC and EC_x for growth rate of algal biomass (cells per volume).

Material and methods:

Test item: Fosetyl-Al, purity: 99.3% w/w, specified by batch no.: OP24550120, TOX no.: 07062-00 and development no.: 3000312323.
The toxicity of the active substance fosetyl-aluminium (fosetyl-Al) to unicellular freshwater alga *Desmodesmus* (*Scenedesmus) subspicatus* was determined over a period of 72 hours. Cultures of alga were exposed to a dilution water control and to nominal test concentrations of the test formulated product (3 replicates of each concentration, 6 replicates of control) equal to 0.954, 3.05, 9.77, 31.3 and 100 mg/L.
Measurements of culture density were made at test initiation (0 hours), at 24 and 48 hours and at test termination (72 hours). Actual concentration of fosetyl-Al present in the test medium was determined at 0 and 73 hours.

Findings:

Test conditions met all validity criteria, given by the guideline mentioned above.
The analytical findings of fosetyl-Al in the treatment levels found on day 0 and 3 were 104 to 110% (average 107%) and 94 to 106% of nominal (average 101%), respectively. All results are based on nominal test concentrations of the test item.
The pH values ranged from 8.0 to 10.6 in the controls and the incubation temperature ranged from 22.0 to 22.6°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6374 lux.

The static 72 hour algae growth inhibition test provided the following effects:

Nominal Concentration [mg a.s./L]	Cell Number after 72 h (means) per mL	(0-72h)-Average Specific Growth Rates [days-1]	Inhibition of Average Specific Growth Rate [%]	Doubling Time of Algae Cells [days]
control	537 000	1.326	--	0.523
0.954	512 000	1.310	1.3	0.529
3.05	520 000	1.317	0.7	0.526
9.77	437 000	1.258	5.1	0.551
31.3	202 000	1.000	24.6	0.693
100	8 000	-0.077	105.8	--

Conclusions:

The 72-h E_rC_{50} for fosetyl-Al is 43.3 mg a.s./L (95 % CI: 31.3 to 56.8 mg a.s./L) and the –72-h NOE_rC is 9.77 mg a.s./L.

CA 8.2.6.2 Effects on growth of an additional algal species

Studies on non-green algae (*e.g.*, the diatom *Navicula pelliculosa*) are not required for fungicides, unless they drive the risk assessment. This is not the case for fosetyl-aluminium (fosetyl-Al), and therefore the following study report, on *Navicula pelliculosa* ([redacted].,; 1988; M-163531-01-1, KCA 8.2.6.2/01), was initially not submitted within the Supplementary Dossier for the EU approval renewal of fosetyl. However, this study was used as supportive information to the BCS response to the request from the RMS about the toxicity of the representative formulation Fosetyl-Al + Fluopicolide WG 71.11 (please refer to the respective Document MCP, Section CP 10.2). Therefore the study summary is provided below; calculation of 72-h EC_{50} is included as it is needed for comparison to the endpoint derived for Fosetyl-Al + Fluopicolide WG 71.11.

Report:	KCA 8.2.6.2/01 [redacted].,; 1988; M-163531-01-1
Title:	The toxicity of fosetyl-al to Navicula pelliculosa.
Report No.:	R002890
Document No.:	M-163531-01-1
Guideline(s):	USEPA (=EPA): J, 123-2, (1982)
Guideline deviation(s):	not specified
GLP/GEP:	**Yes**

Objectives:
The objective of the study was to determine the 7-day EC_{25} and EC_{50} values of fosetyl-aluminium (fosetyl-Al) for *Navicula pelliculosa*. The no observed effect concentration (NOEC) was also determined.

Materials and Methods:
Test item: Fosetyl-Al technical, Batch #DA498, 98.5% (a.s.).
Navicula pelliculosa was exposed over a 7-day period to six concentrations (1.25, 2.5, 5.0, 10, 20 and 40 mg/L nominal) of fosetyl-Al technical and a control. Biomass was determined by cell counts on days 2, 3, 4 and 7.
Percent inhibition, relative to the control, was calculated for each concentration based upon the mean standing crop in cells/mL at 7 days. The EC_{25} and EC_{50} values were determined by weighted least squares nonlinear regression of the log of concentration against cell counts.
The test system consisted of three replicate vessels per test level and per control. The initial cell number was 3000 cells/mL.

The pH values were in the range of 7.5 ± 0.1 in the controls, and the incubation temperature was in the range of 24 ± 2.0 °C over the whole period of testing at a continuous illumination of 4306 ± 646 lux was given.
Samples were analyzed for the actual concentration of fosetyl-Al present in the test solutions on day 0 and at the end of the test.

Results:

Validity criteria:

The study was conducted over a 7-day period. Therefore, cell density at 24 hours is missing. The criteria were therefore checked over a 72-hour period from day 2 to day 4.
Visual inspection of the growth curves shows that there was no inflexion within the 0 to 72 hours period (even until 96 hours) in the control. In addition, over the 0 to 72 hours period, the pattern of toxic effects was already set, with a concentration-response relationship fully consistent with the final result of the study. These warrant the robustness of the comparison between control and exposed algae over the first 72 hours.

Validity criteria (according to OECD 201, adopted 23.03.2006)	**Obtained in this study**
	control
Biomass increased in the control within the evaluation period (criterion is ≥ 16-fold).	40-fold
Mean coefficient of variation for section by section specific growth rates (days 0-1, 1-2, 2-3) in the controls (criterion is ≤ 35%)	10.1%
Coefficient of variation for average specific growth rates during the 0 to 72 hour test period in replicate control cultures (criterion is ≤ 10%).	3.51%

Analytical results:
The mean measured concentrations were 1.58, 2.76, 5.16, 9.27, 17.3 and 28.2 mg/L.

Biological results:
Effects ranged from 29.3% to 99.7% inhibition.

Effect of fosetyl-Al on *Navicula pelliculosa* in a 7 d growth inhibition test

Mean measured concentration [mg a.s./L]	**Cell number after 7 days (means) per mL**	**Inhibition of mean standing crop after 7 days [%]**
Control	3 353 333	--
1.58	2 166 667	35.4
2.76	2 370 000	29.3
5.16	2 060 000	38.6
9.27	1 330 000	60.3
17.3	74 000	97.8
28.2	10 667	99.7

Conclusions:
The 7-day EC_{25} is 6.87 mg/L (95% confidence limits: 5.38 to 8.77 mg/L) and the 7-day EC_{50} is 8.93 mg/L (95% confidence limits: 7.51 to 10.63 mg/L).
The no observed effect concentration (NOEC), based upon the mean standing crop values on day 7, was determined by an analysis of variance and Dunnett's test to be less than 1.58 mg/L.
The 72-h E_rC_{50} recalculated from this dataset using ToxRatPro Version 2.10@ was 18.11 mg a.s./L (95% confidence limits: 14.68 to 22.85 mg/L).

CA 8.2.7 Effects on aquatic macrophytes

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. As an overview the original summary from the DAR is given below.

Report:	KCA 8.2.7/01 [redacted],; 1989; M-163537-02-1
Title:	The toxicity of fosetyl-Al to *Lemna gibba* G3
Report No.:	1163-01-1100-5
Document No.:	M-163537-02-1
Guideline(s):	USEPA (=EPA): J, 123-2
Guideline deviation(s):	none
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

14 d-EC_{50} = 79.67 mg a.s./L

Methods: *Lemna gibba* was exposed over a 14-d period to 6 concentrations of fosetyl-Al (purity: 985 g/kg). Mean measured test concentrations ranged from 12.1 to 305 mg/L. Biomass was determined by frond counts on d 2, 4, 7, 9, 11 and 14.

Results:
Effects ranged from 3.3% to 99.5 % inhibition. Exponential growth was observed at all treatment levels with exception of the two uppermost ones.

EC_{25} – 14-d = 53.07 mg a.s./L (C.I.95%: 43.54 - 64.68 mg a.s./L)
EC_{50} – 14 d = 79.67 mg a.s./L (C.I.95%: 69.52 - 91.39 mg a.s./L)
NOEC – 14 d = 20.4 mg a.s./L.

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary

Material and methods:
Test item: Fosetyl-Al techn. Batch No.: DA498.
Lemna gibba was exposed over a 14-d period under static conditions to the nominal concentrations of 0 (control), 10, 20, 40, 80, 160 and 320 mg a.s./L of fosetyl-Al (purity: 985 g/kg) with three replicates per concentration. At test start 12 fronds were added to each test vessel. Percent inhibition, relative to the control, was calculated for each concentration based upon the mean standing crop in frond numbers at 14 days (i.e. the formula for determination of effects on yield was employed).
The test was performed under continuous light at a temperature of 25 ± 2 °C.

Results:
Analytical findings:
Mean measured test concentrations were 12.1, 20.4, 41.0, 89.4, 162 and 305 mg a.s./L. The results are reported based on the mean measured concentrations (mean of day 0 and day 14).

Biological findings:
Exposure to increasing concentrations of fosetyl-Al had increasingly inhibitory effects upon the population growth of *Lemna gibba* G3. Growth was almost completely inhibited in the two highest concentrations. Effects of the test material on frond number yield on day 14, relative to the control, ranged from 3.3 to 99.5% inhibition.

Frond production recorded for *Lemna gibba* over 14 days exposure to fosetyl-Al

Mean measured concentration [mg a.s/L]	Mean frond number (S.D.)						Percent inhibition
	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14	
Control	25 (0.577)	47 (0.0)	112 (7.37)	189 (11.1)	347 (22)	599 (22.3)	-
12.1	22 (1.0)	42 (1.0)	109 (8.96)	180 (7.55)	389 (33.0)	579 (29.2)	3.3
20.4	24 (1.53)	46 (2.0)	111 (12.4)	175 (17.6)	306 (36.3)	541 (50.8)	9.8
41.0	23 (0.577)	44 (2.89)	100 (7.77)	156 (6.08)	252 (15.9)	472 (34.5)	21.6
89.4	24 (0.577)	43 (2.52)	83 (4.36)	116 (9.07)	170 (5.29)	284 (8.00)	53.6
162	21 (1.15)	27 (2.52)	32 (1.53)	36 (0.577)	39 (3.06)	38 (3.79)	95.5
305	19 (2.0)	22 (1.0)	21 (1.0)	20 (1.53)	18 (0.577)	15 (1.73)	99.5

Request from the RMS:
Further details in the summary of the study of the effects of fosetyl-Al to *Lemna gibba* ([redacted].; 1989; M-163537-02-1) should be provided. A table indicating the percentage of effects and the percentage of inhibition at each observation time would be helpful for a better understanding of the study results. A clear precision on the validity criteria would be also suitable in combination with a justification on the reliability of the study considering the nowadays data requirement for such study (if needed).

Response from BCS:
According to the current guideline OECD 221 (March 2006) the relevant time frame for a growth inhibition study on *Lemna* sp. is 7 days. Accordingly, effects on the measurement variable frond number on the Assessment Days 0, 2, 4, and 7 in this 14-day study ([redacted]P; 1989; M-163537-02-1) are provided in the following. For further details reference is made to the corresponding re-calculation report ([redacted]; 2015; M-525565-01-1) which is provided in KCA 8.2.7/02.

Number of fronds at each assessment day based on arithm. mean measured concentrations

Time	Mean measured conc. [mg a.s./L]						
	Control	12.1	20.4	41.0	89.4	162.0	305.0
Day 0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Day 2	24.7	22.0	23.7	23.3	23.7	20.7	19.0
Day 4	47.0	42.0	46.0	44.3	42.7	26.7	22.0
Day 7	112.3	109.3	111.3	100.3	83.0	32.3	21.0

Inhibition [%] of yield (frond number) at each assessment day based on arithm. mean measured concentrations

Time	Mean measured conc. [mg a.s./L]						
	Control	12.1	20.4	41.0	89.4	162.0	305.0
Day 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Day 2	0.0	21.5	7.89	10.53	7.89	31.58	44.74
Day 4	0.0	14.29	2.86	7.62	12.38	58.1	71.43
Day 7	0.0	2.99	1.0	11.96	29.24	79.73	91.03

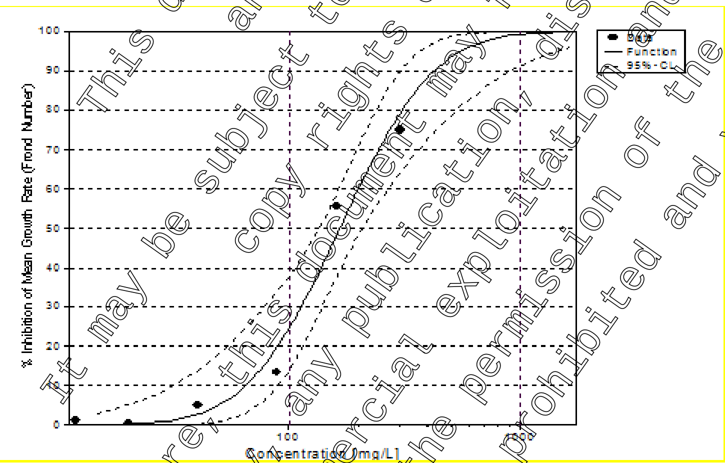
Inhibition [%] of growth rate (frond number) at each assessment day based on arithm. mean measured concentrations

Time	Mean measured conc. [mg a.s./L]						
	Control	12.1	20.4	41.0	89.4	162.0	305.0
Day 0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Day 2	0.0	15.95	5.92	7.72	5.75	24.68	36.72
Day 4	0.0	8.25	1.62	4.38	7.17	41.73	55.69
Day 7	0.0	1.25	0.53	5.08	13.51	55.69	75.0

From the results presented above a $7d\text{-}E_rC_{50}$ = 166.6 mg a.s./L can be derived for frond number based on mean measured concentrations. This figure should be used to address the risk towards aquatic macrophytes as it is in accordance with the requirements of guideline OECD 221 (March 2006) and is in line with current state-of-the-art regarding the preference of growth rate over biomass endpoints.
The *Lemna gibba* study by [redacted]P; 1989; M-163537-02-1 can be regarded as a reliable source of information for the macrophyte risk assessment as it meets the following relevant points:

- the study was conducted according to GLP.
- the validity criterion of OECD 221 for the doubling time of frond number in the control (i.e. seven-fold increase in 7 days) is fulfilled as the factor for frond number increase between day 0 and day 7 was 9.4.
- the amount of substance present in the test medium was analytically determined at the start and at the end of the study; anyhow, all results are based on mean measured concentrations.
- the current guideline OECD 221 asks for a minimum of 5 test item concentrations and a control; in the *Lemna* sp. study by [redacted] 6 test concentrations were tested besides the control.
- as requested by the current guideline OECD 221 three replicates per test level have been used.
- a reliable dose-response can be observed for the effects on growth rate of frond number on day 7 (please see graph directly below and table above).

Dose-response curve showing the influence of the test item on mean growth rate (frond number) of *Lemna gibba* G3 as observed after 7 days



However, it has to be acknowledged that the currently valid guideline OECD 221 asks for data on two measurement variables, i.e. frond no and in addition frond area or dry weight. In the study at hand measurements were performed on the variable frond number only which was in line with the guideline valid at that time (US EPA FIFRA 123-2, 1982).

A recalculation of the endpoints was performed and is submitted within this Supplementary Dossier for the approval renewal of fosetyl. This recalculation is summarized in KCA 8.2.7/02.

Report:	KCA 8.2.7/02[redacted]B; 2015; M-525565-01-1
Title:	Fosetyl-Al (tech.): Recalculation of growth inhibition study with Lemna gibba
Report No.:	M-525565-01-1
Document No.:	M-525565-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Introduction:
A 14-day static *Lemna gibba* growth inhibition study with the test item fosetyl-Al (tech.) has been conducted by [redacted] in 1989 (M-163537-02-1) according to the test guideline valid at that time, *i.e.* US EPA FIFRA 123-2 (1988). This study has been accepted in the original DAR for fosetyl (Feb 2005). However, with regard to the approval renewal of fosetyl, it seems appropriate to recalculate the assessment endpoints for the study at hand based on the current state of science and the currently relevant guideline, i.e. OECD 221 from March 2006.
Even though the current guideline asks for the measurement of 2 variables (frond number and frond area, dry weight or fresh weight), only frond number was measured in this *Lemna* study which is in accordance with the guideline in place at that time. Consequently, this statement presents NOEC, LOEC and EC_{50} values for growth rate after 7 days as calculated by ToxRat Professional, version 2.10.

Material and Methods:
The average specific growth rate (μ) is calculated on the basis of changes in the logarithms of frond numbers for a specified period according to the following equation:

$$\mu = \frac{\ln N_n - \ln N_0}{t_n} \text{ [day}^{-1}]$$

where:
N_0 = nominal number of fronds at time t_0 (test start)
N_n = measured number of fronds after t_n
t_n = elapsed time in days

Percentage inhibition of growth rate μ (Iμ) used in the probit analyses were obtained with the following formula:

$$I\mu = \frac{\mu c - \mu t}{\mu t} * 100\%$$

where:
μ_c = mean growth rate in the control
μ_t = mean growth rate in the treatment group

Results:
The validity criterion stated in OECD 221 (2006) for the doubling time of frond number in the control was fulfilled in the present study. According to the guideline it has to be less than 2.5 days (60 h) which corresponds to approximately a seven-fold increase in seven days. The factor for frond number increase between Day 0 and Day 7 measured in the control of this study was 9.4.
Detailed outputs of the statistical analysis are provided in the Appendix of the report.

The static 7 days growth inhibition test provided the following effects on frond number:

Mean measured test concentration [mg a.s./L]	Frond number (day 7), mean values from 3 replicates	% inhibition of mean growth rate for frond number
control	112.3	--
12.2	109.3	1.25
20.4	111.3	0.53
41.0	100.3	5.08 ●
89.4	83.0	13.51 ●
162	32.3	55.69 ●
305	21.0	75.0 ●

● Results which were significantly different (based on Williams Multiple sequential t-test Procedure) from the control

Conclusion
A summary of the calculated endpoints based on mean measured concentrations is given in the following table:

Endpoint (0-7 day)	Effect on mean growth rate of frond number [mg a.s./L]
E_rC_{50} (CI 95%)	**166.6** (135.1 - 208.3)
LOE_rC	41.0
NOE_rC	20.4

CA 8.2.8 Further testing on aquatic organisms

No further testing on aquatic organisms is deemed necessary due to the results presented in Sections CA 8.2.1 to CA 8.2.7.

Request from the RMS:
The application of fosetyl-Al could expose the non-target organisms to aluminium. The potential impact of Al to the non-target organisms should be discussed and documented. Could you, please, provide further data on this concern?

Response from BCS:
In the EFSA conclusion on the peer review of the pesticide risk assessment of the active substance fosetyl (2013), it was considered that "for soils at pH > 5 (most European cultivated soils), aluminium derived from fosetyl-aluminium (fosetyl-Al) is expected to be immediately adsorbed (ion exchange) and/or rapidly turned into insoluble forms", thus being not available for transfer *via* run-off and drainage to surface water. "For soils at pH < 5, aluminium (Al) derived from fosetyl-Al is expected to join the exchangeable fraction". However, for the intended uses of fosetyl-Al, the concentration that would result in soil is negligible compared to natural exchangeable Al present in soils. Overall, the amount of Al that might be transferred to surface water *via* run-off and drainage is negligible.
Another route of entry of Al into surface water that should also be considered is spray drift. As for soils, the intended uses of fosetyl-Al result in the introduction of negligible amounts of Al^{3+} ions in surface waters. Furthermore, due to its reactivity, the Al^{3+} ion rapidly links to other compounds, so that Al does not occur as free ion in aquatic environments (Ščančar and Milačič, 2006). Complexation of Al in water is influenced by a wide variety of environmental parameters including pH, temperature, dissolved organic carbon, and the nature of the available ligands. In fact, Al chemistry strongly depends on water pH. Above pH 5, Al-hydroxides are the most common Al species in water. Since

95% of European surface waters (n = 3075) with a documented history of exposure to plant protection products fall within the pH range 7.0 - 8.5 ([redacted] *et al*., 2016), Al-hydroxides are the dominant, though labile (Ščančar and Milačič, 2006), water-soluble species of Al in arable landscapes. Other Al species are Al-sulphates and -chlorides, which concentrations depend on the environmental conditions. Al-hydroxides, -chlorides and -sulphates were recently evaluated by ECHA (Registration Dossiers updated in March-April 2016), which has identified no hazard for all these Al species for freshwaters, including sediment.

In conclusion, because of its prominence as a major constituent of the earth's crust, natural weathering processes far exceed the contribution of releases to air, water, and land associated with human activities (Lantzy and MacKenzie, 1979). Accordingly, EFSA conclusion pointed to the fact that "aluminium resulting from the use of fosetyl-Al is expected to have no significant impact on the environment". BCS information concurs to this conclusion.

References:

[redacted] M., [redacted] A., [redacted] M., [redacted] D. and [redacted] R. 2016. Narrow pH range of surface water bodies receiving pesticide input in Europe. *Bull Environ Contam Toxicol*. 96, 3-8.

Lantzy R.J. and MacKenzie, F.T. 1979. Atmospheric trace metals: global cycles and assessment of man's impact. *Geochim Cosmochim Acta* 43, 511-525.

Ščančar J. and Milačič R. 2006. Aluminium speciation in environmental samples: a review. *Anal. Bioanal. Chem*. 386, 999–1012.

Request from the RMS

One of the major metabolite of fosetyl-Al in water system and soil is ethanol. Therefore non-target organisms could be significantly exposed to ethanol. The potential impact of ethanol to the non-target organisms should be discussed and documented. Could you, please, provide further data on this concern?

Response from BCS:

By reference to the most recently updated (April 5, 2016) evaluation of ethanol by ECHA, the $PNEC_{freshwater}$ is 0.96 mg/L (this includes an assessment factor of 10).

On a molecular basis, ethanol is formed as three equivalents from fosetyl-Al. In a worst case situation, considering that fosetyl-Al is transformed instantaneously and completely into ethanol, the ethanol concentration would thus be 55.8 µg/L for the highest fosetyl-Al PEC value derived from a FOCUS Step3 scenario (late application on pome fruit). With a fosetyl-Al PEC value less than 21.3 µg/L (corresponding to the NOEC value derived from the fish ELS study), the ethanol concentration would be 8.3 µg/L.

In the context of the intended uses of fosetyl-Al, the concentration of ethanol resulting from the transformation of fosetyl-Al in water is therefore far below the $PNEC_{freshwater}$ of ethanol. In these conditions, no unacceptable risk for the aquatic environment is to be expected.

This is further supported by the EFSA conclusion on the peer review of the pesticide risk assessment of the active substance fosetyl (2013). According to the EFSA conclusion, "in surface water systems fosetyl-Al degrades rapidly to form ethanol (which also degrades rapidly so is only transient)"; "ethanol is further dissipated by volatilisation or degraded and incorporated in natural constituents of plant and animal tissues".

In conclusion, as a degradation product of fosetyl-Al, ethanol is not expected to adversely affect non-target aquatic organisms.

Request from the RMS:
Following the application of fosetyl-Al, it is expected that a significant amount of phosphate ions will be released in the environment (soil and water). All valuable statements and information about this concern should be provided, especially concerning eutrophication that may occur.

Response from BCS:
In the EFSA conclusion on the peer review of the pesticide risk assessment of the active substance fosetyl (2013), it was considered that the intended uses of fosetyl-Al do not add significant levels of phosphate to soil compared to the amounts naturally present in, or routinely added to, agricultural soils.
With respect to the aquatic environment, the metabolite phosphonic acid (H_3PO_3), in contrast to phosphate (PO_4), is not a macro-nutrient and as such does not contribute to *e.g.* algal blooms. Phosphonic acid and its salts (phosphites) adsorb quickly ($DT_{50,sw}$ = 9.2 days) to the sediment, and are only slowly ($DT_{50,water/sediment}$ = 105 days) oxidized to phosphate under aerobic conditions ([redacted].,; [redacted]; 2005; M-251520-01-1). In this study, it was also shown that no phosphate was formed, probably because bacteria incorporate it for their own growth.
In another context, reference can be made to Council Directive 91/271/EEC concerning urban waste-water treatment. Its objective is to protect the environment from the adverse effects of urban wastewater discharges and discharges from certain industrial sectors. This Directive sets emissions of phosphate to the threshold values of 1,000 to 2,000 µg/L (see Annex III of the Directive). These levels, as continuous emissions, aiming at protecting surface waters from adverse effects, are 10 times higher than the highest phosphate concentrations resulting from fosetyl-Al applications according to the intended uses (FOCUS Step3; pome fruit, 3 x 3.6 kg a.s./ha).
From this information, it can be therefore concluded that, in arable landscapes, fosetyl-Al does not contribute significantly to eutrophication of aquatic ecosystems.

CA 8.3 Effect on arthropods

CA 8.3.1 Effects on bees

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

The following additional studies on toxicity to honey bees and honey bee brood have been performed with technical fosetyl-aluminium (fosetyl-Al) and the formulated product Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) according to current guidelines, guidance documents or the current understanding of the state-of-the-art of testing:

- Chronic 10 day toxicity to adult bees under laboratory conditions,
- Acute contact toxicity to bumble bees under laboratory conditions,
- A colony feeding study following Oomen *et al.* 1992 (using a realistic worse case spray solution concentration and covering exposure for effects on brood (eggs, young and old larvae) and their development, nurse bee on-going behaviour in brood care and colony strength),
- Semi-field brood feeding studies following OECD Guidance Document No. 75 (using a more realistic spray scenario onto flowering *Phacelia* at the maximum application rate for the approval renewal of fosetyl and covering exposure for effects on brood (eggs) and their development and colony parameters).

These studies were not submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC and are submitted within this Supplementary Dossier for the approval renewal of fosetyl. The studies are summarized in the following sections.

Table 8.3.1- 1: EU evaluated and additional studies on bee toxicity of fosetyl-Al and phosphonic acid

Test substance	Test species/ study type	Endpoint	References
Fosetyl-Al	Honey bee, 48 h	LD_{50} – oral > 140 µg a.s./bee LD_{50} – contact > 100 µg a.s./bee	[redacted]; 1997; M-184568-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Honey bee, 48 h	LD_{50} – oral > 462 µg a.s./bee [a)] LD_{50} – contact > 1000 µg a.s./bee	[redacted]; 1999; M-189217-01-1 KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02
	Honey bee, 48 h	LD_{50} – oral > 108.5 µg a.s./bee LD_{50} – contact > 100 µg a.s./bee	[redacted],; 2012; M-440802-01-1 KCA 8.3.1.1.1/04 KCA 8.3.1.1.2/04
Phosphonic acid	Honey bee, 48 h	LD_{50} – oral > 212 µg p.m./bee	[redacted],; 2000; M-238701-01-1 KCA 8.3.1.1.1/03
	Honey bee, 48 h	LD_{50} – contact > 29.7 µg p.m./bee [b)]	[redacted]; 1995; M-179067-01-1 KCA 8.3.1.1.2/03
	Honey bee, 48 h	LD_{50} – oral > 848 µg p.m./bee LD_{50} – contact > 1050 µg p.m./bee	[redacted],; 2010; M-389965-01-1 KCA 8.3.1.1.1/05 KCA 8.3.1.1.2/05
Fosetyl-Al WG 80	Honey bee, 10 d chronic adult feeding study	NOEC 750 mg a.s./kg LC_{50} > 750 mg a.s./kg NOEDD 37.3 µg a.s./bee/day LDD_{50} > 37.3 µg a.s./bee/day	[redacted],; 2015; M-527665-01-1 KCA 8.3.1.2/01
	Honey bee brood feeding (Oomen *et al.*, 1992)	Slightly significantly increased termination rate of eggs, young and old larvae; comparable brood nest development as in control; brood index and brood compensation index displayed continuous increase, indicating a successful development of the brood. No effects on the survival of adult bees and pupae, colony strength and overall colony conditions by feeding honey bee colonies sugar syrup at a fosetyl-Al concentration of 2.4 g a.s./L (2.97 g test item/L).	[redacted],; 2015; M-508986-01-2 KCA 8.3.1.3/01

Test substance	Test species/ study type	Endpoint	References
Fosetyl-Al WG 80	Semi-field honey bee brood study (according to OECD 75; forced exposure conditions) in *Phacelia*; application during full-bloom and bees actively foraging	No adverse effects on mortality, flight intensity, brood development (brood termination rate, brood index, compensation index) as well as on colony strength and brood and food abundance at 3600 g a.s./ha. No adverse effects on mortality, flight intensity, colony strength and brood and food abundance at 570 g a.s./ha.	[redacted].; 2015; M-526896-01-1 KCA 8.3.1.3/02
	Semi-field honey bee brood study (according to OECD 75; forced exposure conditions) in *Phacelia*; application during full-bloom and bees actively foraging	No adverse effects on mortality, flight intensity, behaviour, brood development (brood termination rate, brood index, compensation index) as well as on colony strength and brood and food abundance at 570 g a.s./ha.	[redacted].; 2015; M-528899-01-1 KCA 8.3.1.3/03
Fosetyl-Al	Bumble bee, 48 h	LD_{50} contact > 250 µg a.s./bumble bee	[redacted].; 2015; M-525339-01-1 KCA 8.3.1.1.2/06

p.m. = pure metabolite

a) 96h-endpoint

b) Values were corrected for a purity of 41% phosphonic acid weight by volume which is equal to 29.7% weight by weight. Test substance potassium salts of phosphonic acid has a density of 1.38. Therefore, one L of test substance weighs 1380 g and contains 410 g phosphonic acid (410/1380 = 0.297) with a weight/weight purity of 29.7%.

All studies listed in Table 8.3.1- 1 are summarized in the following sections. In order to facilitate discrimination between new data and data submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC, the old data (summaries from the original DAR prepared by the RMS) are written in grey typeface whereas studies in black typeface are studies of the Supplementary Dossier for the active substance or the representative formulation Fosetyl-Al WG 80.

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

For more information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. As an overview the original summaries from the DAR are given below.

Additional bee acute studies were performed, which were not submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC and are submitted within this Supplementary Dossier for the approval renewal of fosetyl. These studies are summarized below.

Report:	[redacted]$;[redacted];[redacted]; 1997; M-184568-01-1
Title:	Laboratory testing for toxicity (acute contact and oral LD50) of fosetyl-Al to honey bees (Apis mellifera L.) - (Hymenoptera, Apidae)
Report No.:	R011791
Document No.:	M-184568-01-1
Guideline(s):	EPPO: No.170, (1992)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Methods (*acute contact test*):
30 worker honey bees (four to six weeks old) in 3 replicates of 10 were exposed to five dosages of fosetyl-Al (purity: 970 g/kg) ranging from 6.25 to 100 µg/bee over a 48 hour study period.

Results:
Six of the 150 bees (4%) exposed to fosetyl-Al died by the end of the experiment. Mortality occurred in the 6.25 µg and 12.5 µg (both : one dead bee, 3.3%) and in the 25.0 µg and 50.0 µg (both: two dead bees: 6.7%) test substance per bee treatment group. Mortality pattern did not follow a dose-response relationship, because no mortality occurred in the group treated with 100 µg fosetyl-Al per bee. Behavioral abnormalities of a few bees like apathy and moving coordination problems occurred in all groups except in the highest dosage group (100 µg). In the toxic standard (dimethoate) group 29 bees (96.7%). No bee died in the solvent control as well as in the untreated negative control within the experimental observation period.

Methods (*acute oral test*):
30 worker honey bees (10 bees of four to six weeks old per cage) were exposed to five dosages of fosetyl-Al ranging from 8.7 to 140 µg/bee over a 48 hour study period. Acetone was used as a solvent. One solvent (acetone) and a blank control, as well as one toxic standard (dimethoate) test were run in parallel.

Results:
After ingestion of dosages of fosetyl-Al, mortality occurred in the 8.7 µg/bee group only, when two of the 30 bees in this group were found dead (6.7%). No mortality occurred in the other dosage groups. All of the bees in the toxic standard group died within 24 hours of the experimental procedure. In the solvent control, no bees died throughout the course of the experiment.

Contact LD_{50} - 48 h > 100 µg a.s./bee
Oral LD_{50} - 48 h > 140 µg a.s./bee

- **Comments (RMS):** acceptable

Report:	[redacted]4; [redacted]; [redacted]; 1999; M-189217-01-1
Title:	Laboratory testing on the acute contact and oral toxicity of fosetyl-Al to honey bees (Apis mellifera L.), (Hymenoptera, Apidae) - Final report
Report No.:	R014232
Document No.:	M-189217-01-1
Guideline(s):	EPPO: 170, (1992); Equivalent to US EPA OPPTS Guideline No. 850.3020
Guideline deviation(s):	none
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

Contact LD_{50} - 48 h > 1 000 µg a.s./bee
Oral LD_{50} - 96-h = 492 µg a.s./bee

Methods (*acute contact test*):
The study design included 8 experimental groups, each group had 3 replicates with 10 bees per replicate. Fosetyl-Al was applied at the following nominal doses: 2000, 1000, 640, 512 and 410 µg/bee. In addition to the fosetyl-Al-treated groups, one solvent (water and Adhasit) control, one H_2O_2 treated negative control and one positive control with a toxic standard (0.1 µg dimethoate/bee) groups were used in the test.

Methods (*acute oral test*):
The study design included 7 experimental groups, each group had 3 replicates with 10 bees per replicate. Fosetyl-Al (986 g/kg) was applied at the following nominal doses: 1000, 800, 640.0, 512 and 410 µg/bee. The average doses measured were: 1128.3, 882.7, 712.5, 570.9 and 487.9 µg/bee. Duration of the test was extended to 96 h, due to increasing mortality. In addition to the fosetyl-Al-treated groups, one solvent (water and Adhasit, an adherence agent) control and one positive control with a toxic standard (0.2 µg dimethoate/bee) groups were used in the test.

Results:

Contact LD_{50} - 48 h > 1 000 µg a.s./bee

Oral 24-h LD_{50} = 902.4 µg a.s./bee (95% C.I.= 805.1 – 1006.9 µg/bee)
Oral 48-h LD_{50} = 718.8 µg a.s./bee (95% C.I.= 655.0 - 788.9 µg/bee)
Oral 72-h LD_{50} = 542.2 µg a.s./bee (95% C.I.= 471.0 - 624.1 µg/bee)
Oral 96-h LD_{50} = 461.8 µg a.s./bee (95% C.I.= 367.6 - 580.2 µg/bee)

- **Comments (RMS):** Acceptable. Doses higher than 1000 µg/bee were used to cope with potentially high exposure rate.

Report:	KCA 8.3.1.1.1/03 [redacted],; 2000; M-238701-01-1
Title:	Laboratory Testing for Toxicity (Acute oral LD50) of EXP10679A (Potassium salts of Phosphorous acid) on Honeybees (Apis mellifera L.) (Hymenoptera, Apidae)
Report No.:	8341036
Document No.:	M-238701-01-1
Guideline(s):	EPPO: 170 (1992); Equivalent to US EPA OPPTS Guideline No. 850.3020
Guideline deviation(s):	none
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

Oral LD_{50} – 48-h > 212 µg H_3PO_3/bee

Methods:
The test substance EXP10679A (*ca* 401 g phosphorous acid/L) was applied to 2 to 6 weeks old female bees, 10 per test unit. Three replicates per dose level/ were used. EXP10679A was applied in 5 dosages in addition to one solvent control and one positive control with toxic standard (0.2 µg dimethoate/bee). The average dosages ingested in this oral test were: 212.0, 109.0, 55.7, 27.2 and 13.6 µg a.s. per bee. The bees were fed with test item solution, mixed in *ca.* 20 mg/bee ready-to-use syrupto a final sugar solution of 50%.

Results:

Oral LD_{50} – 48 h > 212 µg H_3PO_3/bee

- **Comments (RMS):** acceptable

Report:	[redacted]d; [redacted]; 2012; M-440802-01-1
Title:	Effects of fosetyl - Al tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	73511035
Document No.:	M-440802-01-1
Guideline(s):	OECD 214 and 214 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine the acute contact and oral toxicity of fosetyl-aluminium (fosetyl-Al) tech. to the honey bee (*A. mellifera* L.).
Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and Methods:
Test item: Fosetyl-Al tech.: 98.1% w/w (analysed), Specification No.: 102000016699, Origin Batch No.: 08001, TOX-No: 09393-00.
Under laboratory conditions *Apis mellifera* L. 50 worker bees per dose were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees per dose were exposed for 48 hours for feeding (oral limit test, value based on the actual intake of the test item) to a single dose of 108.5 µg a.s. per bee.

Reference item (nominal dose): 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test); 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test); control: tap water with 0.5% Adhäsit (contact test); 50% sucrose solution (oral test).

Dates of experimental work: May 21, 2012 – May 24, 2012

Results:

Validity criteria:

Validity Criteria	Recommended	Obtained
Control Mortality - Contact Test	≤ 10%	0.0%
Control Mortality - Oral Test	≤ 10%	0.0%
LD_{50} of Reference Item (24 hours) - Contact Test	0.10 – 0.20 µg a.s./bee	0.21 µg a.s./bee
LD_{50} of Reference Item (24 hours) - Oral Test	0.10 – 0.35 µg a.s./bee	0.14 µg a.s./bee

The contact and oral tests are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate), were within the required ranges.

Toxicity to Honey Bees; laboratory tests

Test Item	Fosetyl-Al tech.	
Test Object	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sugar solution)
LD_{50} µg a.s./bee	> 100.0	> 108.5
LD_{20} µg a.s./bee	> 100.0	> 108.5
LD_{10} µg a.s./bee	> 100.0	> 108.5
NOED µg a.s./bee*	≥ 100.0	≥ 108.5

* The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, α = 0.05).

Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	Mean %	Mean %	Mean %	Mean %	Mean %	Mean %
Test item						
100.0	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.30	0.0	6.0	80.0	6.0	84.0	0.0
0.20	0.0	0.0	44.0	8.0	62.0	0.0
0.15	2.0	0.0	22.0	2.0	30.0	0.0
0.10	0.0	0.0	0.0	0.0	0.0	0.0

results are averages from five replicates (ten bees each) per dosage / control
water = CO_2/water-treated control

Mortality and behavioural abnormalities of the bees in the oral toxicity test

Ingested [µg a.s./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality	behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	Mean %	Mean %	Mean %	Mean %	Mean %	Mean %
Test item						
108.5	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.33	20.0	80.0	96.0	0.0	96.0	0.0
0.16	2.0	58.0	66.0	2.0	76.0	0.0
0.08	0.0	2.0	10.0	2.0	14.0	0.0
0.05	0.0	2.0	10.0	2.0	2.0	0.0

results are averages from five replicates (ten bees each) per dosage / control
water = water/sugar treated control

Observations:

Contact Test:
At the end of the contact toxicity test (48 hours after application), no mortality occurred at 100.0 µg a.s./bee. There was no mortality in the control group (water + 0.5% Adhäsit).

Oral Test:
In the oral toxicity test, the maximum nominal test level of fosetyl-Al tech. (*i.e.* 100 µg a.s./bee) corresponded to an actual intake of 108.5 µg a.s./bee. This dose level led to no mortality after 48 hours. In the control group (50% aqueous sugar solution), no mortality occurred.
No test item induced behavioural effects were observed at any time in the contact or oral toxicity tests.

Conclusions:

The toxicity of fosetyl-Al tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 108.5 µg a.s./bee.

Report: [redacted]; [redacted]; 2010; M-389965-01-1
Title: Effects of EXP10679A (potassium salt of phosphorous acid) (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.: 60231035
Document No.: M-389965-01-1
Guideline(s): OECD 213 and 214 (1998)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:

The purpose of this study was to determine the acute contact and oral toxicity of EXP10679A (potassium salt of phosphonic acid) to the honey bee (*A. mellifera* L.).
Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Material and Methods:

Test item: EXP10679A (potassium salt of phosphonic acid): Batch ID.: 2010-005144, TOX09104-00, content of phosphonic acid (AE 0540099): 31.9% w/w, 437.7 g/L (analysed); Synonym: AE 0540099 SL 400 g/L.

Under laboratory conditions *Apis mellifera* 30 worker bees per treatment were exposed for 48 hours to doses of 1050, 583, 324, 180 and 100 µg p.m. (p.m. = pure metabolite) per bee for topical application (contact) and for 48 hours to doses of 848, 719, 577, 411 and 278 µg p.m. per bee for feeding (oral, value based on the actual intake of the test item).

Reference item (nominal dose): 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test); 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test); control: tap water with 0.5% Adhäsit (contact test); 50% sucrose solution (oral test).

Dates of experimental work: August 2, 2010 – August 5, 2010

Results:

Validity criteria:

Validity Criteria	**Recommended**	**Obtained**
Control Mortality - Contact Test	≤ 10%	0.0%
Control Mortality - Oral Test	≤ 10%	0.0%
LD_{50} of Reference Item (24 hours) - Contact Test	0.10 – 0.20 µg a.s./bee	0.20 µg a.s./bee
LD_{50} of Reference Item (24 hours) - Oral Test	0.10 – 0.35 µg a.s./bee	0.19 µg a.s./bee

The contact and oral tests are considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Toxicity to Honey Bees; laboratory tests

Test Item	EXP 10679A (potassium salt of phosphonic acid)	
Test object	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5%)/water)	oral (sugar solution)
Dose [µg p.m./bee]	1050, 583, 324, 180 and 100	848, 719, 577, 411 and 278
LD_{50} [µg p.m./bee]	24 and 48 hrs: > 1050	24 and 48 hrs: > 848

Mortality and behavioural abnormalities of the bees in the contact toxicity test

Dosage [µg p.m./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	Mean %	Mean %	Mean %	Mean %	Mean %	Mean %
Test item						
1050.0	0.0	0.0	0.0	0.0	0.0	0.0
583.0	0.0	0.0	0.0	0.0	0.0	0.0
324.0	0.0	0.0	0.0	0.0	0.0	0.0
180.0	0.0	0.0	3.3	0.0	3.3	0.0
100.0	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.30	0.0	3.3	90.0	3.3	90.0	0.0
0.20	0.0	0.0	50.0	20.0	73.3	3.3
0.15	2.0	0.0	13.3	16.7	30.0	40.0
0.10	0.0	0.0	0.0	3.3	10.0	3.3

results are averages from five replicates (ten bees each) per dosage / control
behav. Abnorm- = behavioural abnormality; water = CO_2/water-treated control

Mortality and behavioural abnormalities of the bees in the oral toxicity test

Ingested [µg p.m./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality	behavioural abnormalities	Mortality	Behavioural abnormalities	Mortality	Behavioural abnormalities
	Mean %	Mean %	Mean %	Mean %	Mean %	Mean %
Test item						
848.0	0.0	50.0	30.0	0.0	33.3	0.0
719.0	0.0	0.0	16.7	3.3	16.7	0.0
577.0	0.0	3.3	10.0	3.3	13.3	0.0
411.0	0.0	0.0	0.0	0.0	3.3	0.0
278.0	0.0	0.0	0.0	0.0	0.0	0.0
water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.31	16.7	40.0	100.0	0.0	100.0	0.0
0.16	0.0	0.0	33.3	13.3	43.3	16.7
0.08	0.0	0.0	0.0	0.0	6.7	0.0
0.06	0.0	0.0	0.0	0.0	3.3	0.0

results are averages from five replicates (ten bees each) per dosage / control
behav. Abnorm- = behavioural abnormalities; water = water/sugar treated control

Observations

Contact Test

Only one single bee was found dead in the 180 µg p.m./bee dose level at test end (48 hours following the application). No bee died after the treatments with any of the other test item dose levels (1050, 583, 324 and 100 µg p.m./bee). No mortality occurred in the control group (water + 0.5% Adhäsit). There were no signs of behavioural abnormalities in any of the dose levels at any time.

<u>Oral Test</u>
In the oral test, the maximum nominal dose levels of the test item (1266 and 844 µg p.m./bee) could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Mortality occurred in the four highest dose levels (848, 719, 577 and 411 µg p.m./bee) in a dose related pattern at the end of the test (48 hours after application). All mortality levels were below 50 %, therefore a LD_{50} could not be determined. No mortality occurred in the 278 µg p.m./bee group and in the control group.
A few single bees in the 848, 719 and 577 µg p.m./bee dose groups were found apathetic during the 4 and 24 hours assessments. 48 hours following the application, no behavioural impairments could be observed anymore.

Conclusions
The toxicity of EXP10679A (potassium salt of phosphonic acid) was tested in both, an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was > 1050 µg p.m./bee in the contact toxicity test. The LD_{50} (48 h) was > 848 µg p.m./bee in the oral toxicity test.

CA 8.3.1.1.2 Acute contact toxicity

For acute contact toxicity on honey bees please refer also to Section CA 8.3.1.1.1.

Report:	KCA 8.3.1.1.2/03 [redacted].; [redacted]; 1995; M-179067-01-1
Title:	Potassium salts of phosphorous acid: An acute contact toxicity study with the honey bee.
Report No.:	394-103-A
Document No.:	M-179067-01-1
Guideline(s):	EPPO: 170; USEPA (=EPA): L: 141
Guideline deviation(s):	none
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

Contact LD_{50} - 48 h > 29.7 µg H_3PO_3/bee

Methods:
Five doses of the test substance (400 g/L) were administered to groups of 60 young adult worker honey bees. Nominal test doses were 6.25, 12.5, 25.0, 50.0 and 100 µg /bee. Solvent control (2 µl of methanol/bee) and positive control tests (dimethoate at 0.05, 0.10 and 0.20 µg/bee) were conducted concurrently.

Results:

Contact LD_{50} - 48 h > 100 µg/bee (> 29.7 µg H_3PO_3/bee)

- **Comments (RMS):** acceptable

An acute contact toxicity study with bumble bees was conducted with fosetyl-aluminium technical.

Report:	KCA 8.3.1.1.2/06 [redacted].; 2015; M-525339-01-1
Title:	Fosetyl-AL technical: Acute contact toxicity to the bumble bee, *Bombus terrestris* L. under laboratory conditions
Report No.:	S14-00625
Document No.:	M-525339-01-1
Guideline(s):	No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010), OECD Guideline 214 (1998), and on the review article of VAN DER STEEN (2001)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:

The objectives of this study were to determine possible effects of fosetyl-aluminium (fosetyl-Al) technical on the bumble bee, *Bombus terrestris* L., from contact exposure and to determine whether the LD_{50} value was greater or lower than the tested dose.

Material and Methods:

Test item: Fosetyl-Al technical: 98.0% (analysed), Batch No.: 201407089, certificate No. AZ 19659.

The test was carried out with young adult worker bumble bees from disease-free and queen-right colonies.

In the laboratory 50 bumble bees (*Bombus terrestris* L.) (5 replicates with 10 individuals) were exposed for 48 hours to a single dose of 250 µg fosetyl-Al/bumble bee by topical application (limit test). In the control group the bumble bees (3 replicates with 10 individuals) were treated by topical application with tap water. The bumble bees of the reference item group were treated with 13 µg dimethoate a.s./bumble bee. Mortality and sub-lethal effects (symptoms of poisoning or any abnormal behaviour in comparison to the control) were assessed 24 and 48 hours after application.

Dates of experimental work: February 3, 2015 – February 5, 2015

Results:

Validity criteria:

The reference item mortality of 93.3% at the end of the test (48 hours after application) was within the required range.

In the control group treated with tap water, no mortality was observed during the 48 hour test period.

Since in the reference item group mortality was ≥ 50% and in the control group mortality was ≤ 10% at the end of the test the test can be considered to be valid.

Contact toxicity to Bumble Bees; laboratory tests

Test Item	Fosetyl-Al tech.
Test Object	*Bombus terrestris*
Exposure	contact
LD_{50} [µg a.s./bumble bee]	> 250.0
LD_{20} [µg a.s./bumble bee]	> 250.0
LD_{10} [µg a.s./bumble bee]	> 250.0
NOED [µg a.s./bumble bee]*	> 250.0

* The NOED was estimated using Fisher Exact Binomial Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Observations:

In the test item treatment group, mortality of 10% was observed at the dose level corresponding to 250 µg fosetyl-Al/bumble bee at the final assessment after 48 hours. In the test item treatment group single affected bees and moribund bees were observed during the entire test period.

Conclusion:
The 48 hour contact LD_{50} value for Fosetyl-Al technical was determined to be > 250 µg fosetyl-Al/bumble bee. The contact NOED (48 h) was determined as 250 µg fosetyl-Al/bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study with honey bees was conducted with Fosetyl-Al WG 80.

Report:	KCA 8.3.1.2/01 [redacted].; 2015; M-527665-01-1
Title:	Fosetyl-AL WG 80H W - Assessment of effects on the adult honey bee, *Apis mellifera* L. in a 10 days chronic feeding test under laboratory conditions
Report No.:	S14-00180
Document No.:	M-527665-01-1
Guideline(s):	based on OECD 213 (1998) and CEB No. 230 with modifications and current recommendations of the ring test group (2014)
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Objective:
The objective of this study was to determine the effects of the test item Fosetyl-Al WG 80 on the adult honey bee, *Apis mellifera* L., in a 10-day chronic feeding test in the laboratory. The Lethal Concentration (LC_{50}), Lethal Dietary Dose (LDD_{50}), No Observed Effect Concentration (NOEC) and the No Observed Effect Dietary Dose (NOEDD) were determined at the end of the test period.

Materials and Methods:
Test item: Fosetyl-Al WG 80, Specification No.: 102000024225-01, Batch ID: EV36003202, Sample description: TOX10146-00, 800 g/kg (nominal), 81% w/w (analysed).
Over a period of 10 days, honey bees were exposed to 50% (w/v) aqueous sucrose feeding solution, with target concentrations of 46.88, 93.75, 187.5, 375 and 750 mg fosetyl-Al/kg feeding solution by continuous and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50% (w/v) aqueous sucrose feeding solution. Mortality and sub-lethal effects were assessed every day throughout the 10-day exposure period. Furthermore, the daily consumption of feeding solution, the mean uptake of test item and the accumulated mean uptake of test item were determined
Samples of the feeding solutions prepared freshly every day throughout the 10-day exposure period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item. During the entire test period the bees were kept under constant darkness except during the assessments.
Reference item (nominal dose): 0.85 mg dimethoate/kg feeding solution, 50% (w/v) sucrose solution.

Dates of experimental work: July 8, 2014 – July 23, 2014

Results:

<u>Validity Criteria</u>
The study is considered valid because:

- The mean mortality in the control was ≤ 15% at the end of the test.
- The mean mortality in the reference item group was ≥50% at the end of the test.

Cumulative mortality, overall mean consumption of feeding solution, dietary dose (DD), accumulated mean uptake of test item as well as the LC_{50}, LDD_{50}, NOEC and NOEDD

Treatment [mg a.s./kg]	10-day cumulative mortality (M_{corr}[4]) [%]	Overall mean consumption of feeding solution [mg/bee/day]	Dietary dose (DD)[5] [µg fosetyl-Al/bee/day]	Accumulated mean uptake of test item [µg fosetyl-Al/bee]
C[1] (0.0)	2.5	43.8	-	-
R[2] (0.85)	57.5 (56.4)	29.2	0.03	0.22
Fosetyl-Al WG 80[3]				
46.88	0.0 (-2.6)	46.9	2.20	22.0
93.75	2.5 (0.0)	46.1	4.32	43.3
187.5	7.5 (5.1)	46.6	8.73	87.4
375	2.5 (0.0)	50.2	18.8	188.1
750	5.0 (2.6)	49.7	37.3	372.6
LC_{50}	> 750 mg a.s./kg			
LDD_{50}	> 37.3 µg a.s./bee/day			
NOEC	750 mg a.s./kg			
NOEDD	37.3 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 Fosetyl-Al WG 80 (a.s. fosetyl-Al)
[4] Corrected morality according to SCHNEIDER-ORELLI (1947)
[5] Dietary Dose (DD): mean uptake of test item (calculation based on the replicate values)

LC Lethal Concentration
LDD Lethal Dietary Dose
NOEC No Observed Effect Concentration based on mortality (not statistically significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $p \leq 0.05$)
NOEDD No Observed Effect Dietary Dose based on mortality (not statistically significantly different compared to the control; Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $p \leq 0.05$)

Observations:
After 10 days of continuous feeding, the mortality at the test item treatment levels of 46.88, 93.75, 187.5, 375 and 750 mg fosetyl-Al/kg feeding solution was not statistically significantly different when compared to the control group.
The cumulative control mortality was 2.5%, as determined at the final assessment after 10 days. The cumulative mortality at the concentration levels of 46.88, 93.75, 187.5, 375 and 750 mg fosetyl-Al/kg feeding solution was 0.0, 2.5, 7.5, 2.5 and 5.0%, respectively (corrected: -2.6, 0.0, 5.1, 0.0 and 2.6%, respectively) at the final assessment.
In the reference item treatment group, mortality increased during the test period and reached 57.5% (corrected 56.4%) after ten days. Exceeding the 50% mortality threshold set as validity criterion, the reference item treatment group showed that the test design is suitable to determine toxic effects in a chronic exposure scenario.
In the control group no sub-lethal effects could be observed. At the concentration level of 375 mg fosetyl-Al/kg feeding solution one affected bee could be observed at assessment E3.

The overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) was not statistically significantly different (lower) when compared to the untreated control group (46.9, 46.1, 46.6, 50.2 and 49.7 mg/bee/day at the concentration levels of 46.88, 93.75, 187.5, 375 and 750 mg fosetyl-Al/kg feeding solution, respectively compared to 43.8 mg/bee/day in the control group). In the toxic reference item group, the overall mean consumption was 29.2 mg/bee/day. At the end of the 10-day exposure period, the mean accumulated uptake of the test item at the concentration levels of 46.88, 93.75, 187.5, 375 and 750 mg fosetyl-Al/kg feeding solution was 21.99, 43.25, 87.36, 188.09 and 372.59 µg fosetyl-Al/bee, respectively (based on the actual consumption of feeding solution by the honey bees). The corresponding daily mean uptake was therefore 2.20, 4.32, 8.73, 18.8 and 37.3 µg fosetyl-Al/bee/day, respectively.

Analytical Results:
The actual concentration of fosetyl-Al in the feeding solutions, determined for each preparation day, was in the range from 73 to 107% of the nominal concentration. The average actual concentration of fosetyl-Al over a period of 10 consecutive days per individual test item treatment level was within the range of 87 to 95% of the nominal concentration. No residues of fosetyl-Al above the LOQ (10 µg/kg) were found in any of the control samples.

Conclusions:
The NOEC for mortality after 10 days of continuous oral exposure was determined to be 750.0 mg fosetyl-Al/kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 37.3 µg a.s./bee/day.
The LC_{50} after 10 days of continuous oral exposure was determined to be > 750 mg fosetyl-Al/kg feeding solution. The corresponding LDD_{50} (Lethal Dietary Dose), based on the actual consumption of the respective feeding solutions, was determined to be > 37.3 µg a.s./bee/day.

Request from the RMS:
A justification of the robustness and the reliability of the NOEC values from chronic toxicity study should be provided. In addition when it is possible, EC10 estimations should also be provided for all chronic studies.

Response from BCS:
For bees one chronic oral feeding laboratory study has been submitted ([redacted]U; 2015; M-527665-01-1). This study was performed as a dose-response test at concentrations ranging between 46.88 and 750 mg a.s./kg (corresponding to 2.2 to 37.3 µg a.s./bee/day). Until the end of the test mortality ranged between 0.0 and 7.5% (corresponding to -2.6 to 5.1% corrected mortality) in the different treatment levels. The highest test concentration of 750 mg a.s./kg that resulted in 5% mortality was determined to be the NOEC (corresponding to 37.3 µg a.s./bee/day being the NOED) since it was not statistically significantly different compared to the control that resulted in 2.5% mortality (Fisher's Exact Test, Bonferroni-Holms corrected, one-sided greater, $p \leq 0.05$). Consequently, since mortality was lower than 10 and 20%, respectively, it is mathematically not possible to calculate LD_{10} and LD_{20} values from the available data.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report:	KCA 8.3.1.3/01 [redacted],; 2015; M-508986-01-2
Title:	Fosetyl-AL WG 80H W - A honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, *Apis mellifera* L. (Hymenoptera: Apidae)
Report No.:	20130047
Document No.:	M-508986-01-2
Guideline(s):	Oomen, P. A., de Ruijter, A. and van der Steen, J. (1992). Method for honeybee brood feeding tests with insect growth-regulating insecticides. EPPO Bulletin, 22, 613-616 [1].
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of the honeybee brood feeding study was to evaluate potential side effects of an application of Fosetyl-Al WG 80 on brood development and mortality of adult worker honeybees (*Apis mellifera* L.).

Materials and Methods:
Test item: Fosetyl-Al WG 80, Specification No.: 102000024225-01, Batch ID: EV36003202, 800 g/kg (nominal); 81% w/w (analysed).
The study included three treatment groups with three replicates (colonies) each: one aqueous sucrose solution treated control group (C), one test-item group (T) and one reference item group (R). The bees were flying freely, with principally unlimited access to natural foraging recourses (e.g., nectar and pollen) in the surroundings. Colonies were set up 7 days before treatment. Applications were made in the evening after flight activity and the colonies were monitored for 22 days. Weather conditions in the pre-application phase were stable with no rain and during post-application phase favourable with rain events 1, 5, 8, 9, 10, 13, 14, 15 and 20 days after treatment (DAT). The application rate of the test item fosetyl-Al WG 80H W was 2.4 g a.s./L. Aqueous sucrose solution was applied at 50% (w/v) in the control group and Insegar 25 WG was applied at 3 g product/L in the reference item group (corresponding to 0.75 g fenoxycarb/L). Treatments were administrated through in-hive feeding of 1 L 50% (w/v) aqueous sucrose solution per colony.
To document the number of dead bees and pupae carried out of the hives, a dead-bee trap was fixed to each hive. For brood development images of entire frames with combs were taken using a Canon EOS 5D Mark II with a macro lens for five runs, each run last for 22 days (developmental cycle of a honeybee worker takes 21 days). One day before application brood areas were fixed (brood area fixing date = BFD) (selection of eggs, young and old larvae for detailed brood assessment). The honeybees were observed until 22 days after treatment. Dead-bee traps were inspected daily and behaviour was also monitored on a daily basis. Food uptake was inspected daily after the start of feeding and lasted until complete consumption of the treatment solution. Colony conditions were assessed two times during the study, specifically on DAT -2 and DAT 22.

The following endpoints were assessed:

- Total and mean mortality of adult worker bees and pupae recorded in dead-bee traps
- Behaviour of bees at the hive entrances
- Colony conditions/performance (brood nest, different brood stages, nectar, honey and pollen stores, and vacant cells)
- Colony strength (number of bees per colony)
- Brood development success (brood termination rate, brood index and compensation index)
- Uptake of feeding solutions

Dates of experimental work: 13 June 2013 – 12 July 2013

Results:

Validity of the Study

The daily mean mortality of adult honey bees (14.26 workers/colony) and pupae (0.65 pupae/colony) in the control treatment during the post-application phase of the study remained low. According to statistical analyses, the overall daily mean pupal mortality in the reference item treatment was significantly higher as compared to the control, indicating that the test system was principally adequate to demonstrate potential effects on honey bee brood.

In addition, there was a statistically significant increase of the mean Brood Termination Rate in the toxic reference treatment for all monitored brood stages on BFD 22 (eggs: 66.67%, young larvae: 40.00%, old larvae: 20.00%) as compared to the control (eggs: 13.33%, young larvae: 3.67%, old larvae: 1.67%). Regarding the overall performance of the reference item and control treatment, the study validity criteria were fulfilled.

Effects of Fosetyl-Al WG 80 on Honeybee Mortality and Honeybee Brood Development

Assessment period	Control n=3	Test Item n=3	Reference Item n=3
	Worker Mortality / Colony and day (Means ± SD)		
Pre-Application (DAT -3 to 0)	18.00 ± 2.88	12.50 ± 4.77	12.33 ± 5.01
Post-Application (DAT 1 to 22)	14.26 ± 2.01	15.24 ± 1.64	9.59 ± 3.92
	Pupal Mortality / Colony (Means ± SD)		
Pre-Application (DAT -3 to 0ba)	0.25 ± 0.25	0.25 ± 0.43	0.75 ± 0.50
Post-Application (DAT 1 to 22)	0.65 ± 0.55	0.44 ± 0.09	34.83 ± 27.06$^{\Delta}$
	Development of selected Eggs (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	13.33 ± 4.73	27.33 ± 13.20$^{\Delta}$	66.67 ± 16.56$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.33 ± 0.24	3.63 ± 0.66	1.67 ± 0.83*
Compensation Index at BFD 22 (DAT 21)	4.44 ± 0.21	3.76 ± 0.70	1.90 ± 0.81*
	Development of selected Young Larvae (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	3.67 ± 1.15	11.33 ± 3.51$^{\Delta}$	40.00 ± 14.73$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.82 ± 0.06	4.43 ± 0.18	3.00 ± 0.74*
Compensation Index at BFD 22 (DAT 21)	4.84 ± 0.08	4.57 ± 0.19	3.04 ± 0.67*
	Development of selected Old Larvae (Means ± SD)		
Brood Termination Rate (%) at BFD 22 (DAT 21)	1.67 ± 1.15	11.00 ± 10.44$^{\Delta}$	20.00 ± 20.88$^{\Delta}$
Brood Index at BFD 22 (DAT 21)	4.89 ± 0.05	4.44 ± 0.52	4.00 ± 1.04
Compensation Index at BFD 22 (DAT 21)	4.93 ± 0.03	4.60 ± 0.40	4.01 ± 1.05

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

In-hive worker mortality (dead bee traps):

No significant differences between treatment groups were detected for the entire post-application phase.

In-hive pupal mortality:

As compared to the control, there was a statistically significant increase of pupae mortality in the reference item throughout the entire post-application phase, but not in the test item.

Behaviour:
In none of the treatment groups abnormal honeybee behaviour was observed during the whole study period.

Colony strength:
There was a significant negative influence on the relative change of the colony strength of the reference item treatment group as compared to the control.

Brood nest (eggs/larvae/pupae):
No significant differences could be detected between treatment groups.

Stores (pollen/nectar/honey):
No significant differences could be detected between treatment groups.

Vacant cells:
No significant differences could be detected between treatment groups.
Brood Termination Rate:
Brood Termination Rates in the test item treatment were statistically significantly higher as compared to the control in all selected brood stages at study end. However, despite the detected statistically significant differences the brood termination rates were generally on a low level with 13.3% (eggs), 3.7% (young larvae) and 1.7% (old larvae) in the control and with 27.3% (eggs), 11.3% (young larvae) and 11.0% (old larvae) in the test item. Also for the reference item all brood stages exhibited statistically significantly increased Brood Termination Rates as compared to the control at study end with 66.7% (eggs), 40.0% (young larvae) and 20.0% (old larvae), showing that the test system was sensitive to detect potential effects of plant protection products on honey bee brood.

Brood Index:
Overall, the Brood Indices of the control and test item displayed comparable increases, indicating a successful development of the brood. Statistical analyses showed that Brood Indices in the test item treatment were not significantly decreased as compared to the control. In contrast, mean brood indices of the reference item treatment for eggs and young larvae were statistically significantly decreased as compared to the control.

Brood Compensation Index
Overall, the Brood Compensation Indices of the control and test item displayed comparable increases, indicating a successful compensation of brood losses. Statistical analyses showed that Brood Compensation Indices in the test item treatment were not significantly decreased as compared to the control. In contrast, the mean Brood Compensation Indices of the reference item treatment for eggs and young larvae were statistically significantly decreased as compared to the control at study end.

Conclusion:
To assess the potential effects of Fosetyl-Al WG 80 on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 2.97 g formulated test item/L (= 2.4 g fosetyl-Al/L) per colony during summer 2013.
The administration of fosetyl-Al WG 80 at a concentration of ~2400 ppm fosetyl-Al to honeybee colonies via feeding of 1 litre spiked sucrose solution has neither resulted in adverse effects on worker or pupal mortality, nor in behavioural abnormalities as compared to the control.
Regarding brood development, Brood Termination Rates of the test item treatment were moderate, yet, as compared to the control, a statistically significant increase was detected at study end, although the Brood Termination Rates in the test item were overall on a low level. However, neither Brood Indices nor Brood Compensation Indices were significantly increased as compared to the control for any brood stage, indicating that these indices performed comparable to the control, including compensations of previous brood losses.

At the level of whole colonies, the performances of overall brood nest size were comparable to the control. Mean colony strength and amounts of stores increased on average and no statistically significant adverse effects were detected at the level of entire colonies.
Despite some short-term effects on Brood Termination Rates, according to the overall results of this study, Fosetyl-Al WG 80 did not adversely affect honeybee colony performance and vitality at a concentration of 2.97 g test item/L (= 2.4 g fosetyl-Al/L).

Report:	KCA 8.3.1.3/02 [redacted].; 2015; M-526896-01-1
Title:	Assessment of side effects of Fosetyl-AL WG 80H W on the honeybee (*Apis mellifera* L.) in the semi-field after one application on *Phacelia tanacetifolia* in Germany 2014
Report No.:	S14-00160
Document No.:	M-526896-01-1
Guideline(s):	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (PISTORIUS *et al*., 2012) OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s):	No major deviations
GLP/GEP:	**yes**

Objective:
This study was designed to determine the potential effects of Fosetyl-Al WG 80 on the honeybee (*Apis mellifera* L.) after one application on *Phacelia tanacetifolia* in Germany in a semi-field brood study.

Materials and Methods:
Test item: Fosetyl-Al WG 80; Specification No.: 102000024225-01, TOX10146-00; Batch-ID: EV36003202; 800 g/kg (nominal); 81.0% w/w (analysed).

The study included four treatment groups with four replicates (tunnels) each: one tap-water treated control group (C), two test-item groups (T1 and T2) and one reference item group (R).
Applications were made at full-flowering (BBCH 63-65) with honeybees actively foraging on the crop. The target application rate of the test item Fosetyl-Al WG 80 in the treatment 1 (T1) was 570 g a.s./ha (actual rate applied 588 g a.s./ha) and in the treatment 2 (T2) 3600 g a.s./ha (actual rate applied 3880 g a.s./ha), respectively. Tap water was applied in the control group and Insegar was applied at a target rate of 1200 g product/ha in the reference item group (corresponding to 300 g fenoxycarb/ha). The spray volume was 400 L/ha in all treatment groups.
The honeybees remained in the tunnels for 12 days and colonies were assessed once before set-up, twice during and four times after the end of the confined phase.

The following endpoints were assessed:

- Total and mean number of dead bees (worker and pupae separately) on the linen sheets in tunnels, in the dead bee traps and in the dead bee bottoms before as well as after the start of exposure in T1, T2 and the application in C and R, respectively.
- Flight intensity (mean number of forager bees/m^2 *Phacelia tanacetifolia*) before as well as after the start of exposure in T1, T2 and the application in C and R, respectively.
- Behaviour of the bees in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony and assessment date).
- Development of the bee brood assessed in individual brood cells. For this particular assessment, between 209 and 262 individually marked cells per colony were selected.

Dates of experimental work: 22 July 2014 – 22 August 2014

Results:

Validity of the Study
The daily mean mortality of pupae (1.7 pupae/colony) in the control treatment during the post-application phase of the study remained low. According to statistical analyses, the overall daily mean pupal mortality in the reference item treatment (17.2 pupae/colony) was significantly higher as compared to the control, indicating that the test system was principally adequate to demonstrate potential effects on honey bee brood.

In addition, there was a statistically significant increase of the mean Brood Termination Rate in the toxic reference treatment at study end. Regarding the overall performance of the reference item and control treatment, the study validity criteria were fulfilled.

Effects of Fosetyl-Al WG 80 on Honeybee Mortality

Treatment group		**Control (C)**	**Test item (T1)**	**Test item (T2)**	**Reference item (R)**
Daily mean mortality (dead worker bees/colony) ± STD	4DBA to 0DBA	57.9 ± 16.0	98.5 ± 36.7	48.3 ± 12.9	62.2 ± 23.0
	0DAA	27.0 ± 6.7	34.8 ± 4.7	21.8 ± 6.5	36.8 ± 10.7
	0DAA to 7DAA	79.3 ± 16.8	106.0 ± 16.7	62.8 ± 24.0	79.6 ± 29.8
	0DAA to 27DAA	34.3 ± 7.5	54.9* ± 16.7	31.3 ± 11.4	34.3 ± 9.8
Daily mean mortality (dead larvae+pupae/colony) ± STD	4DBA to 0DBA	0.8 ± 0.2	2.2 ± 2.6	2.6 ± 3.6	1.2 ± 1.2
	0DAA	2.3 ± 2.2	12.5 ± 23.7	7.5 ± 14.3	1.8 ± 1.5
	0DAA to 7DAA	1.3 ± 0.4	4.2 ± 6.2	3.2 ± 5.5	2.2 ± 2.8
	0DAA to 27DAA	1.7 ± 0.4	3.2 ± 2.8	2.7 ± 3.8	17.2* ± 6.0

DAA: days after application; DBA: days before application; STD: standard deviation
* statistically significantly higher than control group

Throughout the period before exposure, mortality of adult bees across all treatments was similar with exception of test item treatment 1 (T1). In three of four colonies in this treatment, the number of dead adult bees indicated higher sensibility of these colonies to restricted conditions in the tunnels. During exposure from day 0 until day 7 after application, mortality of adult bees across all treatments was similar, indicating no effect of the test item. In the test item treatment 1 (T1) higher mortality values were observed from day 16 until day 22. Since higher mortality in all treatments was observed during this period it could be explained by rainfall that occurred on day 15 and 20. Nevertheless the difference between mean values in the control and T1 in the period 0DAA to 27DAA was statistically significant (Dunnett's t-Test, one sided, $\alpha = 0.05$). No effect of test item treatment 2 (T2) on the mortality of adult bees was observed during the whole exposure period. The number of observed dead pupae and larvae before exposure was similar in all treatments groups. On the day of application higher numbers of dead pupae and larvae were observed in one of the hives of T1 (T1b), the mean value was not statistically higher than the control but this inclination remained until end of exposure period. Since this phenomenon was observed only in one hive, it indicated higher sensibility of this colony to restricted conditions in the tunnel. The mean value of the pupae and larvae mortality in the reference item treatment was statistically significant over the period 0DAA to 27DAA (t-Test pooled, one sided, $\alpha = 0.05$).

Effects of Fosetyl-Al WG 80 on Honeybee Flight Intensity

Treatment group		Control (C)	Test item (T1)	Test item (T2)	Reference Item (R)
Daily mean flight intensity (bees/m^2) ± STD	4DBA to 0DBA	11.7 ± 1.5	12.5 ± 1.0	12.3 ± 1.1	12.5 ± 0.5
	0DAA	14.1 ± 1.2	12.5 ± 1.8	9.3* ± 1.6	6.6* ± 1.6
	0DAA to 7DAA	15.5 ± 1.5	15.0 ± 1.7	14.8 ± 2.8	16.1 ± 0.5

DAA: days after application; DBA: days before application; STD: standard deviation
* statistically significantly lower than control group

Foraging rates were similar across all treatments before exposure (4DBA and 0DBA). On the day of application a short rainfall ca. two hours after the application of reference item occurred. This unfavourable weather condition led to the reduced foraging activity of the bees and was observed during the assessment two hours (2HAA) and four hours (4HAA) after application in R, and four hours (4HAA) after application in C, T1 and T2, respectively. The observations done on 4HAA in all treatment groups and also on 2HAA (only for R) were excluded from the evaluation of the mean values, STD and statistical analysis. On the day of application (0DAA) statistically significantly (Dunnett's t-Test, one sided, α = 0.05) reduced numbers of foraging bees were observed in the test item treatment T2 and reference item treatment (R). From 1DAA to 7DAA foraging activity was similar in all treatments group and no test item and reference item related effects occurred. Thus, no test-item related adverse effects on flight intensity were observed.

Behaviour of the Bees

In the control group bees with locomotion problems, cramping bees and inactive bees were observed during entire study. The abnormal behaviour of the bees described above were more noticeable in the test item group T1 and T2 and reference item group R compared to control group C. Hanging bees, bees clustering at hive, trembling, aggressive to other bees or bees aggressive to observer were additionally observed in T1, T2 and R. Observed abnormal behaviour was related to the exposure phase in the tunnels (0DAA to 7DAA). From day 8DAA some behaviour abnormalities still occurred but were on the similar level as in the control and are not seen as an effect related to the test item.

Development of Honeybee Brood in Individual Cells

Treatment	Brood index / Compensation index at x days after brood area fixing day (BFD)					Termination rate (BFD+21)
	0	+6	+10	+15	+21	[%]
Control	**1.00 / 1.00**	**2.10 / 2.20**	**2.37 / 2.52**	**2.36 / 2.59**	**2.94 / 3.54**	**41.24**
STD	0.00 / 0.00	0.44 / 0.42	0.68 / 0.61	0.68 / 0.57	0.84 / 0.49	16.86
Test item T1	**1.00 / 1.00**	**0.75* / 1.01***	**0.82* / 1.20***	**0.82* / 1.35**	**1.00* / 2.09***	**79.92***
STD	0.00 / 0.00	0.52 / 0.62	0.71 / 0.77	0.71 / 0.99	0.86 / 1.13	17.26
Test item T2	**1.00 / 1.00**	**2.12 / 2.28**	**2.63 / 2.88**	**2.55 / 2.89**	**3.18 / 3.77**	**36.50**
STD	0.00 / 0.00	0.84 / 0.65	1.25 / 0.90	1.21 / 0.80	1.54 / 0.93	30.83
Reference item R	**1.00 / 1.00**	**0.07* / 0.50***	**0.00* / 0.69***	**0.00* / 0.78***	**0.00* / 1.32***	**100.00***
STD	0.00 / 0.00	0.08 / 0.30	0.00 / 0.49	0.00 / 0.56	0.00 / 1.07	0.00

BFD: Brood area fixing day; STD: Standard deviation
*: Statistically significantly lower (brood and compensation indices) or higher (termination rate) compared to the control

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+21) was at 41.24%.
In the test item treatment group T1 the brood development was reduced and mean termination rates were higher than in the control. The mean brood index as well as the mean termination rate on all BFD dates were statistically significantly different from the respective values in the control (Dunnett's t-Test, one-sided, α = 0.05). The mean compensation index was statistically significantly different from the respective values in the control (Dunnett's t-Test, one-sided, α = 0.05) on BFD+21. The mean termination rate at the end of the observation period (BFD+21) was at 79.92%.
In the test item treatment group T2 the brood development and mean termination rates were similar to the control without statistically significant differences. The mean termination rate at the end of the observation period (BFD+21) was at 36.50%.
In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were clearly lower than those observed in the control, indicating a strong adverse effect. The mean brood and compensation indices as well as the mean termination rates in R were statistically significantly different from the respective values in the control on BFD+21s (Satterthwaite t-Test, one-sided, α = 0.05). The mean termination rate at the end of the observation period (BFD+21) was 100.00%, indicating that none of the initially marked eggs had completed its development.

Overall, the quantitative assessments of brood development in individually marked cells revealed that Fosetyl-Al WG 80, applied to full-flowering *Phacelia tanacetifolia* during daily honeybee flight at a rate of 3600 g a.s./ha (test item treatment T2), did not cause any treatment-related adverse effect on honeybee brood development.
The application rate of 570 g a.s./ha (test item treatment T1) gave indication for statistically significantly effects on the brood development. This effect should not be seen as test item related, since three of four colonies in T1 were much more sensitive to restricted conditions in the tunnels than colonies in C and T2. This could be observed in higher mortality of adult bees and pupae already before exposure.
It is known that the assessments of the brood development are a big interference in the condition of the colonies and the method itself can cause irreversible changes to the fragile eggs. The development of the colonies observed during the colony assessment in this treatment was similar to that in the control and no effects on brood were seen on that level.

Strength of the Colonies
No test-item related adverse effects on colony strength were observed.

Development of the Brood Area
Overall, honeybee brood development in the test item treatment groups T1 and T2 was not affected when compared to the control.

Development of the Food Storage Area
No test-item related adverse effects on the development of the food storage area were observed.

Conclusion:
Fosetyl-Al WG 80 was applied at two rates corresponding to 570 g a.s./ha (treatment T1) and 3600 g a.s./ha (treatment T2), at full-flowering *Phacelia tanacetifolia*, during daily honeybee foraging activity. No biologically relevant test-item or rate-response related adverse effects on mortality were observed in T1 and T2. A short-term reduction in foraging activity was seen in T2 and 0DAA. Test-item related effects on behaviour were observed only on the day of application (0DAA) in T1 and T2.
The quantitative assessments of brood development in individually marked cells performed in this study revealed that Fosetyl-Al WG 80 at the rate of 3600 g a.s./ha (T2) did not cause any treatment-related adverse effect on honeybee brood development.

In contrast effects on brood development (termination rates, brood and compensation indices) of Fosetyl-Al WG 80, applied at the lower rate of 570 g a.s./ha (T1) on individually marked cells were observed. The exact reasons for this finding could not be determined. It may be explained by the fact that three out of four colonies in T1 were much more sensitive to restricted conditions in the tunnels than colonies in C and T2 (as confirmed by higher mortality during the pre-exposure phase). Heavy rainfall occurred at least twice during monitoring phase and may have increased sensitivity.
The overall honeybee brood development in the test item treatment groups, measured as mean number of cells covered with the different types of brood per colony was neither affected in treatment T1 nor in T2. Therefore, the effect on brood in T1 was only visible on the level of the development of the individual marked eggs but not on the level of the whole colony.
No test-item related adverse effects on colony strength or on the development of the food storage area were observed in T1 and T2.
Fosetyl-Al WG 80 applied at 570 g a.s./ha to flowering *Phacelia tanacetifolia* in presence of honey bees did not result in test item or rate-response related effects on mortality, flight intensity and colony strength. Effects on brood were seen on the level of the development of individually marked eggs but not on the level of the whole colony.
Fosetyl-Al WG 80, applied at 3600 g a.s./ha to flowering *Phacelia tanacetifolia* in presence of honey bees (T2) resulted in reduced foraging activity on the day of application but did not cause unacceptable effects on mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development.

Request from the RMS:
Further explanations are considered required to conclude on the reliability of the semi-field study in an apple orchard ([redacted].; 2015; M-526896-01-1) for the risk assessment. Could you please indicate if some data are available to precise the level of exposure of the exposed colony? Could you, also, precise if the single application in this study is sufficient to assume that the exposure of bees would be representative of the exposure following the application of the preparation according to the intended GAP (multi-application)?
Without these precisions the reliability of this study could be challenged during the peer-review process.

Response from BCS:
The study by [redacted].; 2015; M-526896-01-1 was not performed in an apple orchard but in *Phacelia tanacetifolia*. This study with specific focus on bee brood development was performed following the OECD Guidance Document 75 that is referenced in the data requirements as set out in Commission Regulation (EU) No 284/2013. The intention is to monitor a complete developmental cycle from egg to adult honeybee. The study design includes a 2 to 3 day acclimatisation period followed by a 7 day direct exposure period after application inside the tunnels under confined conditions before the bees are eventually moved out of the tunnels. Single applications are foreseen by the methodology and in the case of fosetyl-Al, with a minimum application interval of 7 days, multiple applications are virtually impossible to perform when aiming to be compliant with the guidance document. As described in the OECD GD 75 "The bees and their brood are put into an acceptable worst case situation by this test design". It is also stated that "The test chemical has to be applied during full bee flight (e.g., for phacelia, an average of at least 10 bees/m² should be counted at a given time t), to ensure that the colony is exposed to the test chemical." This requirement was completely fulfilled by counts that took place before the application that confirmed the presence of 12.1 bees/m² in the control, 12.9 bees/m² in T1, 11.9 bees/m² in T2 and 12.9 bees/m² in the toxic reference item. Additional evidence on exposure after application is available from the flight assessments that were performed from 15 minutes onwards after the application until day 7 after the application. In the test item treatment groups high foraging activity was found at the majority of timepoints after application (for details, please refer to the study report).

The guidance document also states that a toxic reference item is to be included in the test and the use of at least 150 g fenoxycarb/ha is recommended. As recommended in the guidance document the present study included a toxic reference item applied at 300 g fenoxycarb/ha, which showed the expected effect on brood mortality. The application in all treatment groups was performed with a calibrated portable boom sprayer simulating a commercial application. Several criteria were established on the condition for performance of the application to ensure appropriate exposure. Wind speed was low with 1.0 m/s, no rain occurred within the at least 2 hours after the application and the deviation from the target application rate was +3.22 and +7.77% in the test item and +7.77% in the toxic reference item.

Therefore, based on the available data on monitoring of flight activity, the use of a toxic standard, the technical and meteorological conditions encountered during and after the application, combined with the description of exposure in the guidance document that was followed and that is valid at the time of submission, the applicant is of the opinion that exposure of bees in this study before and after application is sufficiently confirmed and fully compliant with current requirements in place at the time of submission.

In a second study following the same test design clarification was sought on the findings of the lower application rate. The data obtained is presented in the study summary below.

Report:	KCA 8.3.1.3/03 [redacted],; 2015; M-528899-01-1
Title:	Assessment of side effects of fosetyl-AL WG 80H W on the honeybee (*Apis mellifera* L.) in the semi-field after one application on *Phacelia tanacetifolia* in Germany 2015
Report No.:	S15-02966
Document No.:	M-528899-01-1
Guideline(s):	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (PISTORIUS *et al.*, 2012); OEPP/EPPO Guideline No. 170(4) (2010)
Guideline deviation(s):	no major deviations
GLP/GEP:	**yes**

Objective:
This study was designed to determine the potential effects of Fosetyl-Al WG 80 on the honeybee (*Apis mellifera* L.) after one application on *Phacelia tanacetifolia* in Germany in a semi-field brood study.

Materials and Methods:
Test item: Fosetyl-Al WG 80; Specification No.: 102000024225, TOX10146-00; Batch-ID: EV36003889; 800 g/kg (nominal); 80.5% w/w (analysed).

The study included three treatment groups with four replicates (tunnels) each: one tap-water treated control group (C), one test-item group (T) and one reference item group (R).
Applications were made at full-flowering (BBCH 64-65) with honeybees actively foraging on the crop. The target application rate of the test item Fosetyl-Al WG 80 in the treatment (T) was 570 g a.s./ha (actual rate applied 578 g a.s./ha). Tap water was applied in the control group and Insegar was applied at a target rate of 1200 g product/ha in the reference item group (corresponding to 300 g fenoxycarb/ha). The spray volume was 400 L/ha in all treatment groups.
The honeybees remained in the tunnels for 12 days and colonies were assessed twice during and four times after the end of the confined phase.

The following endpoints were assessed:

- Total and mean number of dead bees (worker and pupae) on the linen sheets in tunnels, in the dead bee traps and in the dead bee bottoms before as well as after the start of exposure in T and the application in C and R, respectively.
- Flight intensity (mean number of forager bees/m^2 *Phacelia tanacetifolia*) before as well as after the start of exposure in T and the application in C and R, respectively.
- Behaviour of the bees in the crop and around the hive.
- Condition of the colonies (colony strength and area of the different brood stages and food storage per colony and assessment date).
- Development of the bee brood assessed in individual brood cells. For this particular assessment, between 210 and 253 individually marked cells per colony were selected.

Dates of experimental work: 08 June 2015 – 10 July 2015

Results:

Validity of the Study

The daily mean mortality of pupae (0.2 pupae/colony) in the control treatment during the post-application phase of the study remained low. According to statistical analyses, the overall daily mean pupal mortality in the reference item treatment (25.3 pupae/colony) was significantly higher as compared to the control, indicating that the test system was principally adequate to demonstrate potential effects on honey bee brood.

In addition, there was a statistically significant increase of the mean Brood Termination Rate in the toxic reference treatment at study end. Regarding the overall performance of the reference item and control treatment, the study validity criteria were fulfilled.

Effects of Fosetyl-Al WG 80 on Honeybee Mortality

Treatment group		Control (C)	Test item (T)	Reference item (R)
Daily mean mortality (dead worker bees/colony) ± STD	4DBA to 0DBA	49.2 ± 6.7	45.3 ± 11.3	59.9 ± 10.3
	0DAA	29.8 ± 18.3	19.8 ± 4.4	20.5 ± 6.9
	0DAA to 7DAA	50.3 ± 25.5	39.0 ± 21.7	49.7 ± 41.2
	0DAA to 27DAA	24.5 ± 12.4	18.0 ± 5.0	22.0 ± 13.0
Daily mean mortality (dead larvae+ pupae/colony) ± STD	4DBA to 0DBA	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
	0DAA	0.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.0
	0DAA to 7DAA	0.2 ± 0.2	0.4 ± 0.1	0.5* ± 0.2
	0DAA to 27DAA	0.2 ± 0.2	0.2 ± 0.1	25.3* ± 15.1

DAA: days after application; DBA: days before application; STD: standard deviation

* statistically significantly higher than control group

Throughout the period before exposure, mortality of adult bees across all treatments was similar. During exposure from day 0 until day 7 after application, mortality of adult bees across all treatments was on the same level. The number of dead worker bees in the test item treatment was not statistically significant (t-Test pooled, one sided, $\alpha = 0.05$) in comparison to the control during this period. Throughout the period from 0DAA to 27DAA the number of dead worker bees remained similar across all treatments. Mortality observed in the test item treatment was not statistically significant (t-Test pooled, one sided, $\alpha = 0.05$) in comparison to the control during this period. No effect of test item treatment on the mortality of adult bees was observed during the whole exposure period.

The number of observed dead pupae and larvae before exposure and during exposure in the tunnels was similar in all treatments groups. The mean value of the pupae and larvae mortality in the test item treatment was not statistically significant over the period 0DAA to 27DAA (t-Test pooled, one sided, α = 0.05).
The mean value of the pupae and larvae mortality in the reference item treatment was statistically significantly different over the period 0DAA to 7DAA and 0DAA to 27DAA (t-Test pooled, one sided, α = 0.05).

Effects of Fosetyl-Al WG 80 on Honeybee Flight Intensity

Treatment group		Control (C)	Test item (T)	Reference Item (R)
Daily mean flight intensity (bees/m^2) ± STD	4DBA to 0DBA	12.4 ± 0.7	12.8 ± 2.5	11.8 ± 1.7
	0DAA	15.8 ± 1.7	25.1 ± 5.6	21.8 ± 1.8
	0DAA to 7DAA*	15.4 ± 3.4	21.0 ± 3.2	19.5 ± 1.3

DAA: days after application; DBA: days before application; STD: standard deviation
* Assessments on day 5 and 7 excluded from evaluation due to poor weather conditions (rain) on these days

Foraging rates were similar across all treatments before exposure (4DBA and 0DBA). On the application day the cloudiness changed from 95% (start of application in Ca) to 40% (assessment 15 minutes after the application in Ra) what affected the number of foraging bees: lower rates in the control and higher in the test item and reference item treatments. No test item related effects were observed on that day. From 1DAA to 7DAA foraging activity was similar and no statistically significant (t-Test pooled, one sided, α = 0.05) reduction, in any of the treatments occurred in comparison to the control.

Behaviour of the Bees
Abnormal behaviour such as locomotion problems, cramping, trembling, inactive bee, hanging bees and intensive cleaning were observed throughout the duration of the study in all treatments. On few occasions unusual behaviour was observed in T from day of application until the end of the study. Often comparable behaviour was also observed in the control. Overall the number of affected bees in the test item was always very low (max. 24 bees in all test item tents on the day of application).

Development of Honeybee Brood in Individual Cells

Treatment	Brood index / Compensation index at x days after brood area fixing day (BFD)					Termination rate (BFD+21)
	0	+6	+10	+16	+21	[%]
Control	**1.00 / 1.00**	**2.35 / 2.36**	**2.87 / 2.92**	**2.82 / 3.09**	**3.52 / 4.11**	**29.63**
STD	0.00 / 0.00	0.50 / 0.50	0.59 / 0.64	0.66 / 0.35	0.83 / 0.25	16.45
Test item T	**1.00 / 1.00**	**2.25 / 2.26**	**2.62 / 2.65**	**2.58 / 2.74**	**3.20 / 3.68**	**36.11**
STD	0.00 / 0.00	0.71 / 0.72	1.00 / 1.03	0.96 / 1.01	1.24 / 1.26	24.79
Reference item R	**1.00 / 1.00**	**0.44* / 0.44***	**0.15* / 0.37***	**0.10* / 1.43***	**0.12* / 2.91***	**97.63***
STD	0.00 / 0.00	0.35 / 0.34	0.19 / 0.26	0.11 / 0.91	0.14 / 0.83	2.74

BFD: Brood area fixing day; STD: Standard deviation
*: Statistically significantly lower (brood and compensation indices) or higher (termination rate) compared to the control

In the control group C, successful development was observed in the majority of the marked brood cells, indicating a healthy development of brood. The mean termination rate at the end of the observation period (BFD+21) was at 29.63%.
In the test item treatment group T the brood development and mean termination rates were similar to the control without statistically significant differences. The mean termination rate at the end of the observation period (BFD+21) was at 36.11%.
In the reference item treatment group R, the post treatment mean values of the brood and compensation indices were clearly lower than those observed in the control, indicating a strong adverse effect. The mean brood and compensation indices as well as the mean termination rates in R were statistically significantly different from the respective values in the control for all post treatment assessments (Satterthwaite t-Test, one-sided, $\alpha = 0.05$). The mean termination rate at the end of the observation period (BFD+21) was 97.63%, indicating that the most of the initially marked eggs hadn't completed its development.

Overall, the quantitative assessments of brood development in individually marked cells revealed that Fosetyl-Al WG 80, applied to full-flowering *Phacelia tanacetifolia* during daily honeybee flight at a rate of 570 g a.s./ha (test item treatment T), did not cause any treatment-related adverse effect on honeybee brood development.

Strength of the Colonies
No test-item related adverse effects on colony strength were observed.

Development of the Brood Area
Overall, honeybee brood development in the test item treatment groups T was not affected when compared to the control.

Development of the Food Storage Area
The majority of the colonies were well provided during the course of the study. Thus, no test-item related adverse effects on the development of the food storage area were observed.

Conclusion:
Fosetyl-Al WG 80 was applied at a target rate corresponding to 570 g a.s./ha (treatment T), at full-flowering *Phacelia tanacetifolia*, during daily honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of brood and brood cell development were evaluated. No test-item related adverse effects on mortality were observed. No reduction in foraging activity was seen in T throughout the study. On few occasions unusual behaviour was observed in T. Often comparable behaviour was also observed in the control. Overall the number of affected bees in the test item was always very low.
The quantitative assessments of brood development in individually marked cells performed in this study revealed that Fosetyl-Al WG 80 at a target rate of 570 g a.s./ha did not cause any treatment-related adverse effect on honeybee brood development. No test-item related adverse effects on colony strength or on the development of the food storage area were observed in T.
Fosetyl-Al WG 80 applied at a target rate of 570 g a.s./ha to flowering *Phacelia tanacetifolia* in presence of honey bees did not result in test item related effects on mortality, flight intensity, colony strength and brood development.

Request from the RMS:
Further explanations are considered required to conclude on the reliability of the semi-field study in an apple orchard ([redacted], B.; 2015; [redacted],; 2015; M-528899-01-1) for the risk assessment. Could you please indicate if some data are available to precise the level of exposure of the exposed colony?
Without these precisions the reliability of this study could be challenged during the peer-review process.

Response from BCS:
The study by [redacted],; 2015; M-528899-01-1 was not performed in an apple orchard but in *Phacelia tanacetifolia*. This study with specific focus on bee brood development was performed following the OECD Guidance Document 75 that is referenced in the data requirements as set out in Commission Regulation (EU) No 284/2013. The intention is to monitor a complete developmental cycle from egg to adult honeybee. The study design includes a 2 to 3 day acclimatisation period followed by a 7 day direct exposure period after application inside the tunnels under confined conditions before the bees are eventually moved out of the tunnels. Single applications are foreseen by the methodology and in the case of fosetyl-Al, with a minimum application interval of 7 days, multiple applications are virtually impossible to perform when aiming to be compliant with the guidance document. As described in the OECD GD 75 "The bees and their brood are put into an acceptable worst case situation by this test design". It is also stated that "The test chemical has to be applied during full bee flight (e.g., for phacelia, an average of at least 10 bees/m² should be counted at a given time t), to ensure that the colony is exposed to the test chemical." This requirement was completely fulfilled by counts that took place before the application that confirmed the presence of 11.3 bees/m² in the control, 15.5 bees/m² in the test item and 15.0 bees/m² in the toxic reference item. Additional evidence on exposure after application is available from the flight assessments that were performed from 15 minutes onwards after the application until day 7 after the application. On the majority of timepoints after application (for details, please refer to the study report) even higher foraging activity than before the application was seen.
The guidance document also states that a toxic reference item is to be included in the test and the use of at least 150 g fenoxycarb/ha is recommended. As recommended in the guidance document the present study included a toxic reference item applied at 300 g fenoxycarb/ha, which showed the expected effect on brood mortality. The application in all treatment groups was performed with a calibrated portable boom sprayer simulating a commercial application. Several criteria were established on the condition for performance of the application to ensure appropriate exposure. Wind speed was low with 1.4 m/s, no rain occurred within the at least 2 hours after the application and the deviation from the target application rate was +1.43% in the test item and +0.41% in the toxic reference item.
Therefore, based on the available data on monitoring of flight activity, the use of a toxic standard, the technical and meteorological conditions encountered during and after the application, combined with the description of exposure in the guidance document that was followed and that is valid at the time of submission, the applicant is of the opinion that exposure of bees in this study before and after application is sufficiently confirmed and fully compliant with current requirements in place at the time of submission.

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.

CA 8.3.2 Effects on non-target arthropods other than bees

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience.

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

CA 8.3.2.2 Effects on Typhlodromus pyri

Studies on non-target arthropods have been performed with the representative formulations and are presented in the respective Document MCP, Section 10.3.2.

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 for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The study from which the endpoint will be used for risk assessment is summarised below from the original DAR of fosetyl.

In order to address new data requirements according to Regulation (EC) No 1107/2009, an additional study on chronic exposure to earthworms with Fosetyl-Al WG 80 has been performed and is submitted within this Supplementary Dossier for the approval renewal of fosetyl.

Table 8.4.1- 1: Ecotoxicological endpoints – additional earthworm reproduction studies with active substance fosetyl-Al and its metabolite

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Fosetyl-Al WG 80	*Eisenia fetida* reproduction 56 d, mixed	NOEC	316 mg prod./kg dws 254.4 mg a.s./kg dws	[redacted],; 2015; M-531997-01-1 KCA 8.4.1/02
Phosphonic acid	*Eisenia fetida* Reproduction, 56 d, mixed	NOEC	≥498.79 mg pm/kg dws a)	[redacted],; 1999; M-189218-01-1 KCA 8.4.1/01
	Eisenia fetida Reproduction, 56 d, mixed	NOEC	<693 mg pm/kg dws	[redacted],; 2009; M-327177-01-1 KCA 8.4.1/03

dws = dry weight soil; a.s. = active substance; prod. = product; pm = pure metabolite
grey typeface = study is part of the Baseline Dossier

a) Values were corrected for a purity of 41.8% phosphonic acid weight by volume which is equal to 29.9% weight by weight. Test substance potassium salts of phosphonic acid has a density of 1.397. Therefore, one L of test substance weighs 1397 g and contains 418 g phosphonic acid (418/1397 = 0.299) with a weight/weight purity of 29.9%.

All studies listed in the Table 8.4.1-1 are summarized below.

In order to facilitate discrimination between new data and data submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC, the old data (summaries from the original DAR prepared by the RMS) are written in grey typeface whereas studies in black typeface are studies of the Supplementary Dossier for the active substance fosetyl-aluminium or the representative formulation Fosetyl-Al WG 80.

Report:	KCA 8.4.1/01 [redacted].; 1999; M-189218-01-1
Title:	Effects of EXP10679A (Potassium salts of phosphorous acid) on reproduction and growth of earthworms Eisenia fetida (Savigny 1826) in artificial soil
Report No.:	R014233
Document No.:	M-189218-01-1
Guideline(s):	BBA: VI, 2-2, (1994); ISO: 11268-2, (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:

NOEC = 1667 mg product/kg soil

Methods:

A total of 360 adult earthworms adults approximately 6 months old with an individual bodyweight at start of the test ranging from 331 to 568 mg (10 adults per replicate, 4 replicates per experimental group) were used in the study. There were 9 experimental groups: a control, 7 concentrations of the test substance and a toxic standard (carbendazim at 2.62 mg/kg soil). The nominal concentrations of EXP10679A (418 g/L) were 26, 52, 104, 208, 417, 833 and 1 667 mg/kg soil (equivalent to 7.78, 15.56, 31.12, 62.24, 124.77, 249.24 and 498.79 mg phosphonic acid/kg soil, respectively). Chemical analysis to measure actual concentrations was not conducted.

Results:

In the control group, two adult worms were found dead in one replicate resulting in an overall mortality of 5%. No mortality occurred in any of the groups exposed to the test substance or to the toxic standard.
From the start to the end of the test, adult earthworms gained weight in the control and all test substance groups. Bodyweight increase in the control was 9.6% and in the test substance groups ranged from 10.7 to 24.7%. There were no statistically significant differences between the control and the five lowest test substance concentrations (26, 52, 104, 208 and 417 mg/kg). The bodyweight increase observed in the two highest concentrations of the test substance (833 and 1 667 mg/kg) was significantly higher than in the control.
No behavioural or morphological abnormalities were observed in adult worms exposed to the test substance. As the potential effect observed in these two groups was bodyweight increase rather than decrease, these changes should not be taken into account to establish the NOEC value. Worms significantly lost weight in the toxic standard group.
The mean number of offspring produced in each of the test substance-treated groups (ranging from 331 to 460) did not differ statistically from that of the control group (464). The number of juveniles in the toxic standard group was very low (13) and significantly different than in the control.
Food consumption was not affected by the treatment.

EC_{10} cannot be calculated, since the data do not indicate a dose response. The data meet the guideline requirements (coefficient of variation of the control reproduction <30%). The NOEC is therefore considered reliable.

NOEC = 1667 mg/kg soil (nominal concentration)
(499 mg H_3PO_3/kg soil; nominal concentration)

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Current Guideline:
OECD. (2004), *Test No. 222: Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris

Test endpoints according to current guideline:

- Mortality
- Weight change
- Reproduction

Exposure according to current guideline:
Test containers made of glass or other chemically inert material of about one to two liters capacity should be used. The containers should have a cross-sectional area of approximately 200 cm² so that a moist substrate depth of about 5 to 6 cm is achieved when 500 to 600 g dry mass of substrate is added. The design of the container cover should permit gaseous exchange between the substrate and the atmosphere and access to light (e.g. by means of a perforated transparent cover) whilst preventing the worms from escaping. If the amount of test substrate used is substantially more than 500 to 600 g per test container the number of worms should be increased proportionately. A solution of the test substance in de-ionised water is prepared immediately before starting the test in a quantity sufficient for all replicates of one concentration. A co-solvent may be required to facilitate for the preparation of the test solution. It is convenient to prepare an amount of solution necessary to reach the final moisture content (40 to 60% of maximum water holding capacity). The solution is mixed thoroughly with the soil substrate before introducing it into a test container. The test containers are first filled with the moistened soil substrate and the weighed worms are placed on the surface.

Evaluation according to current guideline:
On day 28 the living adult worms are removed from the test vessels, counted and weighed. Any worms not found at this time are to be recorded as dead. If the soil has been removed from the containers it is then returned (minus the adult worms but containing any cocoons that have been produced). The soil is then incubated for four additional weeks under the same test conditions except that feeding only takes place once at the start of this phase of the test. At the end of the second 4-week period, the number of juveniles hatched from the cocoons in the test soil and cocoon numbers are determined. All signs of harm or damage to the worm should also be recorded throughout the test period.

Validity Criteria:

	Guideline	Test result
Number of juveniles produced in each replicate (containing 10 adults) until the end of the study for the control	≥ 30	464
Coefficient of variation of reproduction on the control	≤ 30%	13.1%
Adults mortality over the initial 4 weeks on the control	≤ 10%	5%

Study Remarks:
No major differences were found between the current guideline and the developed study. However, there were some small and not significant variations observed. The temperature values increased for 1 and for 7 hours by 2 °C compared to the upper limit (22 °C) stated in the guideline. This minor deviation is not expected to have a significant influence of the study results.

Conclusion:
The test design of the actual study is in line with the requirements of the current guideline. The validity criteria of the current test guideline were fulfilled.
Due to the fact that no dose response was observed and no mortality occurred, EC_x could not be calculated.

Report:	KCA 8.4.1/02 [redacted]; 2015; M-531997-01-1
Title:	Fosetyl-Al WG 80 W: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No.:	15 10 48 143 S
Document No.:	M-531997-01-1
Guideline(s):	OECD 222 (2004); ISO 11268-2 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine the sublethal effects of the test item on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil in a laboratory test.

Materials and Methods:
Test item: Fosetyl-Al WG 80, Short name: FEA WG 80, Supplier batch No.: EV36003889, Sample description: TOX10884-00, Specification No.: 102000024225, active ingredient (analysed content): 80.5% w/w fosetyl-aluminium (LS 74783), water solubility: dispersible.

Adult earthworms (*Eisenia fetida*, about 3 months old, 8 × 10 animals for the control group and 4 × 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg soil dry weight (d.w.). The test item was mixed into the soil.
Artificial soil composition was 69.5% quartz sand, 20% kaolin clay, 10% sphagnum peat and 0.5% $CaCO_3$. The vessels were kept in a temperature-controlled room at 19.0 to 22.0 °C under a 16-hour light to 8-hour darkness photoperiod and a light intensity at light period of approximately 530 lux. Earthworms were fed with dried horse manure.
After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Toxic standard: 5 and 10 mg Nutdazim 50 FLOW/kg soil d.w.; control: untreated, solvent control: none.

Findings:

Effects on mortality, growth and reproduction of the earthworms

Test item **Test object** **Exposure**	**Fosetyl-Al WG 80** ***Eisenia fetida*** **Artificial soil**		
	Mortality	**Biomass change**	**Reproduction**
	[mg test item/kg d.w.]		
NOEC LOEC	≥ 1000 > 1000	≥ 1000 > 1000	316 562
EC_{10} [1)] (95% confidence limits)	-	-	360 (294 – 439)
EC_{20} [1)] (95% confidence limits)	-	-	459 (396 – 531)

[1)] based on Probit analysis

Observations:

	Fosetyl-Al WG 80 [mg test item/kg d.w.]								
	Control	**18**	**32**	**56**	**100**	**178**	**316**	**562**	**1000**
Mortality of adult worms after 4 weeks									
Mortality (%)	1.3	0.0	0.0	2.5	0.0	0.0	0.0	2.5	5.0
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)									
Mean (mg)	128.3	145.7	131.1	124.9	147.4	141.9	134.4	135.6	127.8
Mean (%)	33.3	37.7	34.1	32.4	38.0	36.4	34.9	35.2	33.1
Number of juveniles per surviving adult worm after 8 weeks									
Mean	15.8	15.3	15.9	16.2	15.7	15.7	16.2	10.1	5.0
Number of juveniles per replicate after 8 weeks									
Mean	155.0	152.5	158.8	158.0	157.0	156.8	162.0	98.5*	48.0*
Reproduction compared to control (%)									
% to control	100	98.4	102.4	101.9	101.3	101.1	104.5	63.5	31.0

No statistically significant differences between the control and test item were calculated for mortality (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, $\alpha = 0.05$, one-sided greater)
* statistically significantly different compared to control for biomass and reproduction (Williams-t-test, $\alpha = 0.05$, one-sided smaller)

The mortality of adult worms was 0 to 5.0% in the treated groups and 1.3% in the control group. No statistically significant mortality compared to the control was observed at any test item concentration (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, $\alpha = 0.05$, one-sided greater). No pathological symptoms and no effects on behaviour (including feeding activity) of the worms were observed during the test.
The weight change of adult worms ranged between 33.1 and 38.0% in the treated groups and was 33.3% in the control group. The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams-t-test, $\alpha = 0.05$, one-sided smaller).
Statistically significant effects (Williams-t-test, $\alpha = 0.05$, one-sided smaller) on number of juveniles compared to the control group were recorded at concentrations of 562 and 1000 mg test item/kg d.w.

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	1.3%
Number of juveniles per replicate	≥ 30	156, 125, 173, 166, 134, 187, 130, 169
Coefficient of variation of reproduction	≤ 30%	14.7%

All validity criteria for the study were met.

In a reference test, the number of juveniles was reduced by 46 and 100% by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) at concentrations of 5 and 10 mg/kg d.w. in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

EC_{10} value has been reported and has been included in the summary. The data meet the guideline requirements (coefficient of variation of the control reproduction < 30%). The NOEC is therefore considered reliable.

Conclusions:
Fosetyl-Al WG 80 showed no statistically significantly adverse effects on mortality and biomass of the earthworm *Eisenia fetida* in artificial soil up to and including 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested. The test item showed statistically significantly adverse effects on reproduction at 562 and 1000 mg test item/kg soil d.w.

Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 316 mg test item/kg soil d.w.,
and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 562 mg test item/kg soil d.w.

Report: KCA 8.4.1/03 [redacted]., 2009; M-327177-01-1
Title: Fosetyl-Al: Effects of the soil metabolite of fosetyl-Al, phosphite, on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No.: LRT-RG-R-54/09
Document No.: M-327177-01-1
Guideline(s): ISO 11268-2: 1998 (E) and OECD 222; April 13, 2004
Guideline deviation(s): Aim of the study was to test the effects of phosphite on the survival, growth and reproduction of Eisenia fetida. In this study phosphite is formed by the degradation of fosetyl-Al in soil. Therefore the fosetyl-Al was mixed into artificial soil 7 days before the earthworms were added to the test system.
GLP/GEP: **yes**

Objective:
The purpose of this study was to assess the effect of the soil metabolite of fosetyl-aluminium (fosetyl-Al), phosphite, on survival, growth, and reproduction on the earthworm *Eisenia fetida* during an exposure into an artificial soil at 4 different test concentrations.

Materials and Methods:
Test item: Fosetyl-Al (Specification No.: 102000002957; Article No.: 05930170; Batch Code: AE F053616-01-07; Origin Batch No.: PF90205140; TOX-No.: 08344-00; content of a.s. (analysed): 99.6% w/w).

Fosetyl-Al was mixed into artificial soil (10% peat) at nominal test concentrations of 1100, 1980, 3366 and 5722 mg/kg dwt soil 7 days before the test organisms, adult *Eisenia fetida* (approx. 8 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration) were added. During an incubation period of seven days fosetyl-Al (DT_{50} 0.125 days) rapidly degrades to form the major soil metabolite phosphite [equivalent to phosphonic acid] (DT_{50} 119 days). The nominal test concentrations of the active substance correspond to nominal amount of phosphite formed of 693, 1247, 2121 and 3605 mg phosphonate/kg dwt soil, confirmed analytically.

After 28 days of exposure the earthworms were removed after counting of survivors and noting their weights. After further 28 days, the number of offspring was determined.

Findings:
Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days.

Test object	***Eisenia fetida***				
	Control	**Treatment Groups**			
Nominal concentration of fosetyl-Al [mg/kg dwt soil]	---	1100	1980	3366	5722
Nominal concentration of phosphite [mg/kg dwt soil]	---	693	1247	2121	3605
Analytical verified amount of phosphite [mg/kg dwt soil]	---	731	1374	2162	3531
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%]	+ 52.9	+ 56.7	+ 50.5	+ 26.5	- 5.3
Standard Deviation	± 8.5	± 5.6	± 5.2	± 5.9	± 10.6
Statistical comparison to the control [1)]	---	n.s.	n.s.	s.	s.
Mean number of offspring per test vessel after 56 days	246.9	95.8	34.3	21.0	0.0
Standard Deviation	± 27.4	± 48.7	± 13.9	± 9.5	± 0.0
Statistical comparison to the control [2)]	---	s.	s.	s.	s.

1) Result of a Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$
2) Result of a Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$
n.s.: mean value not statistically significant different compared to the control ($p \geq 0.05$)
s.: mean value statistically significant different compared to the control ($p < 0.05$)

Observations:
No mortality of adult earthworms was observed after 28 days of exposure at any test concentration of the test item in this study.

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 1100 and 1980 mg a.s. fosetyl-Al/kg dry weight artificial soil equivalent to 693 and 1247 mg a.s. phosphite/kg dry weight artificial soil. Statistically significant different values for the growth relative to the control were observed at the test concentrations of 3366 and 5722 mg a.s. fosetyl-Al/kg dry weight artificial soil equivalent to 2121 and 3605 mg a.s. phosphite/kg dry weight artificial soil.

Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentration of 1100, 1980, 3366 and 5722 mg a.s. fosetyl-Al/kg dry weight artificial soil equivalent to 693, 1247, 2121 and 3605 mg a.s. phosphonate/kg dry weight artificial soil.

Validity Criteria	Recommended	Obtained
Adult mortality	≤ 10%	0.0%
Number of juveniles per replicate	≥ 30	246.9 (control)
Coefficient of variation of reproduction	≤ 30%	11.1%

All validity criteria for the study were met.

Calculation of an EC_{10} value is not considered appropriate since the effect on reproduction was in the range of 61 to 100%. No NOEC was derived from these study results.

Before start of the study, the nominal application rates of the test item were calculated based on the amount of dry soil. The samples taken for analytical verification of the concentration of phosphonic acid were in wet (humid) state and were extracted in that state. However, the humidity of the samples was determined.
The recoveries given in Appendix V Table A5 (report page 50; see column 'actual concentration of phosphorous acid' 'wet soil [%]) were by mistake calculated by comparing the concentrations based on wet soil with the nominal rates based on dry soil which results in wrong recovery values.
The correct nominal and the analyses residue values based on dry soil are reported in section 2.4.4 on page 19 of the report. Based on the residue values (related to the dry weight soil) the correct recovery values have now been calculated and are presented in the table below.

Nominal test concentration mg a.s. fosetyl-Al/kg dws*	**Nominal test concentration** mg a.s. phosphite/kg dws*	**Analysed test concentration** mg a.s. phosphite/kg dws*	**Recovery** Percentage of nominal test concentration
1100	693	731	**105%**
1980	1247	1374	**110%**
3366	2121	2162	**102%**
5722	3605	3531	**98%**

* dws = Dry weight artificial soil

The new calculated recovery values based on the dry weight soil residue values showed that the analyzed concentrations of phosphonate confirm the nominal test concentration in the test system. A GLP amendment of the report with a corrected version of Table A5 in Appendix V has been initiated and can be provided on request.

Conclusions:
The NOEC for growth was 1980 mg fosetyl-Al equivalent to 1247 mg phosphite/kg dwt soil.
The NOEC for reproduction was < 1100 mg fosetyl-Al equivalent to < 693 mg phosphonate/kg dwt soil [equivalent to < 693 mg phosphonic acid/kg dwt soil], the lowest concentration tested.

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

No studies were submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC, therefore testing on springtails (*Folsomia candida*) and soil mites (*Hypoaspis aculeifer*) was performed with the representative formulation Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) and the metabolite phosphonic acid. The corresponding summaries are provided below in Section CA 8.4.2.1.

Table 8.4.2- 1: Ecotoxicological endpoints – Collembola and soil mites reproduction studies with active substance fosetyl-Al and its metabolite

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Collembola, reproduction				
Fosetyl-Al WG 80	*Folsomia candida* reproduction 28 d, mixed	NOEC	562 mg prod./kg dws 452.4 mg a.s./kg dws	[redacted]P; 2015; M-529932-01-1 KCA 8.4.2.1/01
Phosphonic acid	*Folsomia candida* reproduction 28 d, mixed	NOEC	≥ 1000 mg pm/kg dws	[redacted],; 2015; M-529267-01-1 KCA 8.4.2.1/03
Soil mites, reproduction				
Fosetyl-Al WG 80	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	≥ 1000 mg prod./kg dws ≥ 805 mg a.s./kg dws	[redacted],; 2015; M-531417-01-1 KCA 8.4.2.1/02
Phosphonic acid	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	≥ 1000 mg pm/kg dws	[redacted]r; 2015; M-532897-01-1 KCA 8.4.2.1/04

dws = dry weight soil; a.s. = active substance; pm = pure metabolite, prod. = product

CA 8.4.2.1 Species level testing

Report: KCA 8.4.2.1/01 [redacted],; 2015; M-529932-01-1
Title: Fosetyl-Al WG 80 W: Effects on the reproduction of the collembolan *Folsomia candida*
Report No.: 15 10 48 141 S
Document No.: M-529932-01-1
Guideline(s): OECD 232 (2009), ISO 11267 (1999)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective

The purpose of this study was to determine potential effects of different concentrations of Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted.

Material and methods:

Test item: Fosetyl-Al WG 80, Short name: FEA WG 80, Supplier batch No.: EV36003889, Sample description: TOX10884-00, Specification No.: 102000024225, active ingredient (analysed content): 80.5% w/w fosetyl-aluminium (LS 74783), water solubility: dispersible.

10 *Collembola* (9-12 days old) were exposed to 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$, at 19.2 to 22.0 °C and a photoperiod: light : dark = 16 h : 8 h (540 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg soil d.w; control: untreated; solvent control: none.

Findings:

Mortality:
Mortality rates of 2.5 to 10.0% were recorded in the test item treatment groups. 2.5% parental mortality was observed in the control. No statistically significant effect (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) on parental mortality was found for any concentration tested. No effects on behaviour of the collembolans were observed during the test.

Reproduction:
The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 1072 in the control and 1063, 1073, 1065, 1083, 1041, 1075, 1004 and 909 at concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg soil d.w., respectively. Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group was recorded at a concentration of 1000 mg test item/kg soil d.w.

Test item Test object Exposure	**Fosetyl-Al WG 80 *Folsomia candida* Artificial soil**						
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles per test vessel ± standard deviation**			**Reproduction (% of control)**	**Significance (*)**
Control	2.5	1072	±	107	-	
18	2.5	1063	±	195	99	-
32	2.5	1073	±	82	100	-
56	2.5	1065	±	115	99	-
100	2.5	1083	±	142	101	-
178	5.0	1041	±	46	97	-
316	5.0	1075	±	76	100	-
562	2.5	1004	±	175	94	-
1000	10.0	909	±	246	85	+
					Reproduction	
$NOEC_{reproduction}$ (mg test item/kg soil dry weight) $LOEC_{reproduction}$ (mg test item/kg soil dry weight)					562 1000	
					Reproduction	
EC_{10} (mg test item/kg soil dry weight) [1)] 95% confidence limits EC_{20} (mg test item/kg soil dry weight) [1)] 95% confidence limits					774 (676 – 887) 1191 (999 – 1420)	

The calculations were performed with unrounded values
[1)] Logit analysis
(*) = (Williams-t-test one-sided-smaller, α = 0.05, + = significant, - = not significant)
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated groups
R_c = mean number of juveniles observed in the control group

Validity of the study:
Validity criteria for the untreated control of the study according to OECD 232 (2009).

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20%	2.5%
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1072
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	10.0%

All validity criteria were met. Therefore this study is valid.

In a separate study (BioChem project No. R 15 10 48 003 S, dated August 04, 2015), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 103 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

EC_{10} value has been reported and has been included in the summary. The data meet the guideline requirements (coefficient of variation of the control reproduction < 30%). The NOEC is therefore considered reliable.

Conclusion:
Fosetyl-Al WG 80 showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at concentrations up to and including 1000 mg test item/kg d.w.
Fosetyl-Al WG 80 caused a significant reduction of reproduction of the collembolan *Folsomia candida* in artificial soil at 1000 mg test item/kg soil dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 562 mg Fosetyl-Al WG 80/kg soil d.w. and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 1000 mg Fosetyl-Al WG 80/kg soil d.w.

Report:	KCA 8.4.2.1/02 [redacted], 2015; M-531417-01-1
Title:	Fosetyl-Al WG 80 W: Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*
Report No.:	15 10 48 142 S
Document No.:	M-531417-01-1
Guideline(s):	OECD 226 (2008)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to determine potential effects of Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (Canestrini) as a representative of soil micro-arthropods during a test period of 14 days.

Materials and Methods:
Test item: Fosetyl-Al WG 80, batch No.: EV36003889, Sample description: TOX10884-00, Specification No.: 102000024225, analytical findings: 80.5 % w/w fosetyl-aluminium (LS 74783).

Ten adult, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control (water treated) and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/ kg dry weight soil were tested. In each test vessel 20 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (35 days after start of egg laying). During the test, they were fed every 2 - 3 days with *Tyrophagus putrescentiae* (Schrank).

During the study a temperature of 19.7 to 21.9 °C and light regime of 523 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand, 5% sphagnum peat, 0.2 % $CaCO_3$ and 20% kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing liquid. All *Hypoaspis aculeifer* were counted.

Findings:

Validity Criteria	Recommended	Obtained
Mean mortality of adult females	≤ 20%	6.3%
Mean number of juveniles per replicate	≥ 50	262.5
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	12.9%

All validity criteria for the study were met.

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item **Test object** **Exposure**	**Fosetyl-Al WG 80** ***Hypoaspis aculeifer*** **Artificial soil**	
	Adult mortality	**Reproduction**
	(mg test item/kg soil d.w.)	
NOEC	≥ 1000	≥ 1000
LOEC	> 1000	> 1000

EC_{10} and EC_{20} values could not be determined due to mathematical reasons.

Reference test:
In a separate study (BioChem project No. R 14 10 48 001 S, dated June 10, 2014), the EC_{50} (reproduction) of the reference item Dimethoate was calculated to be 6.2 mg/kg soil d.w.
The results of the reference test demonstrate the sensitivity of the test system.

Observations:

Endpoint		**Treatment group (mg test item/kg soil d.w.)**				
	Control	**100**	**178**	**316**	**562**	**1000**
Mortality of soil mites after 14 days (%)	6.3	0.0	7.5	0.0	2.5	2.5
Mean number of juveniles after 14 days	262.5	288.5	282.8	266.0	314.8	301.0
CV (%)	12.9	13.0	7.7	21.5	4.8	6.1
Reproduction (% of control)	**100**	**110**	**108**	**101**	**120**	**115**

Not statistically significantly different compared to the control (Chi[2] 2x2 Table Test with Bonferroni Correction for mortality, $\alpha = 0.05$, one-sided greater; Welch-t-test for Inhomogeneous Variances with Bonferroni-Holm Adjustment for reproduction, $\alpha = 0.05$, one-sided smaller)
Calculations were done using unrounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group
CV (%) = Coefficient of variation

Mortality:
In the control group a parental mortality of 6.3% could be observed. The mortality in the test item treatment groups ranged between 0.0 and 7.5%.

Reproduction:
Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 262.5 in the control and 288.5, 282.8, 266.0, 314.8 and 301.0 at concentrations of 100, 178, 316, 562 and 1000 mg test item/kg soil d.w., respectively.

EC_{10} cannot be calculated, since the exposure to test item did not result in an adverse effect on reproduction. The data meet the guideline requirements (coefficient of variation of the control reproduction < 30%). The NOEC is therefore considered reliable.

Conclusion:
The test item Fosetyl-Al WG 80 showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at all tested concentrations.
Therefore, the No-Observed-Effect-Concentration (NOEC) and Lowest-Observed-Effect-Concentration (LOEC) for mortality and for reproduction were determined to be ≥ 1000 and > 1000 Fosetyl-Al WG 80/kg soil d.w., respectively.

Report:	KCA 8.4.2.1/03 [redacted]; 2015; M-529267-01-1
Title:	Dipotassium phosphonate (BCS-CZ96503): Effects on the reproduction of the collembolan *Folsomia candida*
Report No.:	15 10 48 206 S
Document No.:	M-529267-01-1
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
Guideline deviation(s):	none
GLP/GEP:	yes

Objective
The purpose of this study is to determine potential effects of different concentrations of dipotassium phosphonate (salt of phosphonic acid) on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans were counted.

Material and methods:
Test item: Dipotassium phosphonate (BCS-CZ96503), Batch code: BCS-CZ96503-PU-01, Origin Batch No.: SES 12947-1-1, Certificate No.: AZ 20059, LIMS No.: 1510480, CAS No.: 13492-26-7, analytical findings: 98.5% w/w.

10 *Collembola* (9 to 12 days old) were exposed to 27, 48, 85, 100, 178, 316, 562 and 1000 mg pure substance/kg dry weight of soil containing 74.7% quartz sand, 20% kaolin clay, 5% sphagnum peat and 0.3% $CaCO_3$, at 18.0 to 22.0 °C and a photoperiod: light : dark = 16 h : 8 h (530 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44, 67, 100, 150 and 225 mg boric acid/kg dws; control: untreated, solvent control: none.

Findings:

Mortality:
Mortality rates of 0 to 7.5% were recorded in the test item treatment groups. 5.0% parental mortality was observed in the control. No statistically significant effect (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, α = 0.05, one-sided greater) on parental mortality was found for any concentration tested. No effects on behaviour of the collembolans were observed during the test.

Reproduction:
The mean number of juvenile collembolans counted four weeks after introduction of the parental collembolans into the test vessels was 1037 in the control and 1031, 1037, 1034, 1065, 1000, 1013, 1025 and 1037 at concentrations of 27, 48, 85, 100, 178, 316, 562 and 1000 mg pure substance/kg dws, respectively. No statistically significant effect (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles was found for any concentration tested.

Test item **Test object** **Exposure**	**Dipotassium phosphonate (BCS-CZ96503)** ***Folsomia candida*** **Artificial soil**			
mg pure substance/kg dry weight soil nominal concentration	**Adult mortality (%)**	**Mean number of juveniles per test vessel ± standard deviation**	**Reproduction (% of control)**	**Significance (*)**
Control	5.0	1037 ± 128	-	
27	0.0	1031 ± 139	99	-
48	5.0	1037 ± 202	100	-
85	7.5	1034 ± 143	100	-
100	5.0	1065 ± 74	103	-
178	5.0	1000 ± 89	96	-
316	7.5	1013 ± 62	98	-
562	2.5	1025 ± 87	99	-
1000	2.5	1061 ± 122	102	-
$NOEC_{reproduction}$ (mg pure substance/kg soil dry weight)			≥1000	
$LOEC_{reproduction}$ (mg pure substance/kg soil dry weight)			>1000	

The calculations were performed with unrounded values
(*) = (Williams-t-test one-sided-smaller, α = 0.05, + = significant, - = not significant)
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juveniles observed in the treated groups
R_c = mean number of juveniles observed in the control group

Validity of the study:
Validity Criteria for the untreated control of the study according to OECD 232 from September 07, 2009.

Validity criteria	**Recommended by the guideline**	**Obtained in this study**
Mean adult mortality	≤ 20%	5.0%
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1037
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30%	12.3%

All validity criteria were met. Therefore this study is valid.

In a separate study (BioChem project No.: R 14 10 48 003 S, dated July 30, 2014), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 104 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

EC_{10} cannot be calculated, since the exposure to test item did not result in an adverse effect on reproduction. The maximum deviation from the control was < 10%. The data meet the guideline requirements (coefficient of variation of the control reproduction < 30%). The NOEC is therefore considered reliable.

Conclusion:
Dipotassium phosphonate (salt of phosphonic acid) showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at concentrations up to and including 1000 mg pure substance/kg dws. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg pure substance/kg dws, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg pure substance/kg dws.

Report:	KCA 8.4.2.1/04 [redacted]; 2015; M-532897-01-1
Title:	Dipotassium phosphonate (BCS-CZ96503): Influence on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested in artificial soil
Report No.:	E 428 4713-9
Document No.:	M-532897-01-1
Guideline(s):	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: Not Applicable; OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The purpose of this study was to assess the effect of dipotassium phosphonate (salt of phosphonic acid) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment.

Materials and Methods:
Test item: Dipotassium phosphonate (BCS-CZ96503) (analytical findings: 98.5% w/w (BCS-CZ96503); batch code: BCS-CZ96503-PU-01; certificate no.: AZ 20059; origin batch no.: SES 12947-1-1).
Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg pure substance/kg dry weight artificial soil were tested.
During the test, the *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. During the study a temperature of 20 ± 2 °C and light regime of 400 to 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75% fine quartz sand, 5% Sphagnum peat, air dried and finely ground, 20% Kaolin clay.
After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen apparatus. Extracted mites were collected in a fixing solution. All *Hypoaspis aculeifer* were counted under a binocular.

Findings:

Validity Criteria	Recommended	Obtained
Mean mortality of adult females	≤ 20%	2.5%
Mean number of juveniles per replicate	≥ 50	205.6
Coefficient of variation (mean number of juveniles per replicate)	≤ 30%	12.9%

All validity criteria for the study were met.

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Dipotassium phosphonate (BCS-CZ96503) *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg pure substance /kg soil d.w.)	
NOEC	≥ 1000	≥ 1000
LOEC	> 1000	> 1000

The $EC_{10, 20}$ -values could not be determined due to mathematical reasons.

Reference test:
The most recent non-GLP-test ([redacted], LAR/HR-O-16/14, January 05, 2015) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.
Dimethoate EC 400E G showed an EC_{50} of 5.47 mg a.s./kg (95% confidence limits from 4.09 mg a.s./kg to 7.30 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression.
This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 to 7.0 mg a.s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Observations:

Test item Test object Exposure	Dipotassium phosphonate (BCS-CZ96503) *Hypoaspis aculeifer* Artificial soil				
mg pure substance/Kg dry weight artificial soil	Adult mortality (%)	Significance (*)	Mean number of juveniles per test vessel ± standard dev.	Reproduction (% of control)	Significance (**)
Control	2.5	---	205.6 ± 26.6	---	---
100	0.0	-	234.3 ± 15.1	113.9	-
178	0.0	-	246.8 ± 13.3	120.0	-
316	0.0	-	244.8 ± 12.3	119.0	-
562	2.5	-	247.3 ± 31.3	120.2	-
1000	0.0	-	266.0 ± 23.7	129.4	-

Calculations were done with un-rounded values.
(*) = Fisher´s exact Binomial Test with Bonferroni Correction, one-sided-greater, α=0.05, “-“: non-significant; “+”: significant
(**) = William's-t.-test, one sided smaller; α=0.05; “-“: non-significant; “+”: significant

Mortality:
In the control group 2.5 of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20% mortality.
Concerning the mortality of the adult test organisms statistical analysis (Fisher´s Exact Binomial Test with Bonferroni Correction, one-sided greater, α = 0.05) revealed no significant difference between control and any treatment group.

Reproduction:
Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group.

EC_{10} cannot be calculated, since the exposure to test item did not result in an adverse effect on reproduction. The data meet the guideline requirements (coefficient of variation of the control reproduction < 30%). The NOEC is therefore considered reliable.

Conclusion:
Overall NOEC: ≥1000 mg pure substance/kg dry weight artificial soil
Overall LOEC: >1000 mg pure substance/kg dry weight artificial soil

CA 8.5 Effects on soil nitrogen transformation

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The study from which the endpoint will be used for risk assessment is summarised below from the original DAR of fosetyl.

Additional N-transformation studies were performed, which were not submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC and are submitted within this Supplementary Dossier for the approval renewal of fosetyl. These studies are summarized below.

Table 8.5- 1: Studies on nitrogen transformation with fosetyl-Al and its metabolite

Test substance	Test species/study type	Endpoint	References
Fosetyl-Al	Study duration 28 d	no unacceptable effects	20.0 kg a.s./ha 26.6 mg a.s./kg dws	[redacted]; 1998; M-184321-01-1 KCA 8.5/01
Fosetyl-Al WG 80	Study duration 42 d	no unacceptable effects	987 kg prod./ha 1304 mg prod./kg dws 1067 mg a.s./kg dws	[redacted]; 2008; M-307736-01-1 KCA 8.5/02
Phosphonic acid	Study duration 42 d	no unacceptable effects	48.98 kg pm/ha **65.31 mg pm/kg dws**	[redacted]T; 2015; M-528580-01-1 KCA 8.5/03

dws = dry weight soil; a.s. = active substance; pm = pure metabolite; prod. = product
grey typeface = study is part of the Baseline Dossier

Report: KCA 8.5/01 [redacted]; 1998; M-184321-01-1
Title: A laboratory assessment of the effects of fosetyl-Al on soil microflora respiration and nitrogen transformations according to current EU guidelines.
Report No.: R001660
Document No.: M-184321-01-1
Guideline(s): EPPO Bulletin 24, 111, (1994)
Guideline deviation(s): not specified
GLP/GEP: yes

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for fosetyl-Al:
No significant effect (±25%) at 20 kg a.s./ha

Methods
A solution of fosetyl-Al (purity: 993 g/kg) was applied to either a low and high organic matter loamy sand soil (according to Dutch standard NEN5795; BBA-German classification: both loamy sand soils; ADAS-UK classification: both sandy loam soils) at a concentration of 26.6 mg/kg assuming 100% distribution in the soil with a bulk density of 1.5 g/cm^3 to a depth of 5 cm (equivalent to a concentration in the soil following direct application of 20 kg/ha).

The effects of fosetyl-Al on microbial respiration were investigated using short term respiration experiments conducted after 0, 13 (15 in the case of the humus loamy sand), and 28 d. On each occasion, aliquots of soil were amended with a non-limiting quantity of glucose and carbon dioxide evolution measured over the subsequent 24 h period. The effects on nitrogen transformations, ammonification and nitrification were investigated in soil amended with ground lucerne grass. An annual test with a toxic reference (dinoseb acetate) was performed.

Results:
A small amount of ammonium was sometimes detected (control: 0.09 ppm and 2 x field rate treated: 0.07 ppm, respectively). This leads to a high percentage deviation. However, this percentage deviation was not found to be significant at 5% level.
No statistically significant effects greater than ± 25% of control values on soil microflora respiration and soil nitrogen transformations.

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Current Guideline:
OECD. (2000), *Test No. 216: Soil Microorganisms: Nitrogen Transformation Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.

Test endpoints according to current guideline:
- Nitrate formation rate

Exposure according to current guideline:
Test containers made of chemically inert material are used. They should be of a suitable capacity in compliance with the procedure used for incubation of soils, i.e. incubation in bulk or as a series of individual soil samples. Care should be taken both to minimise water loss and to allow gas exchange during the test (e.g. the test containers may be covered with perforated polyethylene foil). Containers should be of a size such that approximately one quarter of their volume is filled with the soil sample. The soil is divided into three portions of equal weight. Two portions are mixed with the carrier containing the product, and the other is mixed with the carrier without the product (control). A minimum of three replicates for both treated and untreated soils is recommended. Care should be taken to ensure homogeneous distribution of the test substance in the treated soil samples. During mixing, compacting or balling of the soil should be avoided.

Evaluation according to current guideline:
Soil samples are analyzed for nitrate on days 0, 7, 14 and 28. Nitrate is extracted from soil by shaking samples with a suitable extraction solvent, e.g. a 0.1 M potassium chloride solution. The mixtures are centrifuged or filtered and the liquid phases are analyzed for nitrate. The rate of nitrate formation in treated samples is compared with the rate in the controls, and the percent deviation of the treated from the control is calculated. All tests run for at least 28 days. If, on the 28th day, differences between treated and untreated soils are equal to or greater than 25%, measurements are continued to a maximum of 100 days. If a prolonged test is required, further measurements should be made at 14 days intervals after day 28. The amount of nitrate formed in each treated and control replicate is determined at each sampling time.

Validity Criteria:

	Guideline	Test result	
		Soil sample	
		98/047/03	*98/063/03*
Variation between replicate control samples	< 15%	33.5% (13 days)	3.5 (28 days)

Study Remarks:

No major differences were found concerning the design and conduct between the current guideline and the actual study with the exception that according to the current guideline, when analysing agro-chemicals, two different concentration rates should be tested. In the study here present, only one concentration was used, nevertheless the test item rates used in this study were higher than the field used rate, and therefore can be considered acceptable for risk assessment.

The study here presented was evaluated according to an older guideline, thus, the evaluations were based on the nitrate concentrations at the different points in time and not based on the nitrate formation rates.

The reported data have been re-analysed for the tested soils based on the reported data to derive the nitrate formation rates as requested in the current guidelines. It could be concluded that the results for the soil 98/047/02 does not match the new validity criteria since the variation between replicate control samples on day 13 exceeded with an actual value of 33.5% the acceptable limit of 15%. For this reason the analyses of this soil were not considered for further evaluations. Detailed results for this study re-analyses for soil 98/047/02 are presented in the table below.

Days after treatment	Control		26.6 mg/kg	
	mg nitrogen / kg dry weight soil			
	Sample	nitrate	Sample	nitrate
0	1	13.1	1	11.2136
	2	12.8904	2	13.0470
	3	14.41	3	12.9428
	4	15.8772	4	14.934
	MV	**14.07**	MV	**13.03**
	±	±	±	±
	SD	1.38	SD	1.52
	CV	9.8%		
13	1	12.4712	1	16.9776
	2	12.3664	2	20.0168
	3	13.9384	3	14.2004
	4	23.2132	4	10.3752
	MV	**15.50**	MV	**15.39**
	±	±	±	±
	SD	5.19	SD	4.10
	CV	***33.5%***		
28	1	35.4748	1	17.1872
	2	26.7764	2	32.6976
	3	32.6976	3	18.2352
	4	26.9336	4	31.0732
	MV	**30.47**	MV	**24.80**
	±	±	±	±
	SD	4.33	SD	8.22
	CV	14.2%		

A re-assessment of the results for the second soil (98/065/03, see table below) indicate that the criteria in the currently guideline are met, and therefore can be considered valid for the re-evaluation.

Days after treatment	Control		26.6 mg/kg	
	mg nitrogen / kg dry weight soil			
	Sample	nitrate	Sample	nitrate
0	1	19.9786	1	19.1418
	2	19.4033	2	17.4682
	3	20.6585	3	18.9326
	4	19.7694	4	17.2067
	MV	**19.95**	MV	**18.19**
	±	±	±	±
	SD	0.53	SD	0.99
	CV	2.6%		
13	1	44.3073	1	41.84
	2	40.5848	2	40.5325
	3	37.1853	3	36.61
	4	42.7814	4	41.6831
	MV	**41.26**	MV	**40.17**
	±	±	±	±
	SD	3.16	SD	2.44
	CV	7.7%		
28	1	71.2326	1	69.7682
	2	68.513	2	69.4544
	3	66.4072	3	68.4084
	4	66.3164	4	65.1658
	MV	**68.04**	MV	**68.20**
	±	±	±	±
	SD	2.39	SD	2.10
	CV	3.5%		

An assessment of the nitrate formation rates for the second soil (98/065/03) is presented below.

Time interval (days)	control		26.6 mg/kg		
	mg nitrogen / kg dry weight soil / time interval / day				
	Sample	nitrate	Sample	nitrate	% Diff.
0-13	1	1.89	1	1.75	
	2	1.63	2	1.77	
	3	1.27	3	1.36	
	4	1.77	4	1.88	
	MV	**1.64**	MV	**1.69**	3.13
	±	±	±	±	
	SD	0.27	SD	0.23	
13-28	1	1.78	1	1.86	
	2	1.86	2	1.93	
	3	1.93	3	2.12	
	4	1.57	4	1.57	
	MV	**1.79**	MV	**1.87**	4.69
	±	±	±	±	
	SD	0.16	SD	0.23	

Conclusion:
A re-assessment of the results for the second soil (98/065/03, see table above) indicate that the criteria in the currently guideline are met, and therefore can be considered valid for the re-evaluation.
The re-assessment of the second soil (98/065/03) concerning the nitrate formation rates indicated that it meets the current guideline requirements. A deviation percentage of 3.13% in the nitrate formation rate was observed in the first 13 days after treatment between the treatment and the control. Afterwards, from day 13 to day 28, a percentage deviation of 4.69% was found for the same parameter. Therefore, fosetyl-Al applied at a rate of 26.6 mg a.s./kg soil did not deviate in excess of ± 25% from the control concerning the nitrate formation rate after 28 days.

Report:	KCA 8.5/02 [redacted]Z; 2008; M-307736-01-1
Title:	Fosetyl-Al WG 80C W: Determination of effects on nitrogen transformation in soil
Report No.:	LRT-N-106/08
Document No.:	M-307736-01-1
Guideline(s):	OECD 216; adopted January 21, 2000; OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test.
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The objective of the test was to determine the influence of 130.4 mg and 1304.0 mg of Fosetyl-Al WG 80/kg dry weight soil on nitrogen transformation in an agricultural soil.

Materials and Methods:
Test item: Fosetyl-Al WG 80 (analytical finding: 81.8% w/w, specification No.: 102000001579, batch No.: EV38000066, TOX-No.: FAR01374-00).
A silty sand soil was exposed for 42 days to 130.4 mg and 1304.0 mg product/kg dry weight soil by mixing into the soil. Application rates were equivalent to 97.8 kg and 978 kg test item/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.

Findings:
The coefficient of variation in the control at the end of the study was 2%. Therefore the validity criteria for the study, which requires a coefficient of variation ≤ 15% in the control was fulfilled.

Effects on non-target soil microorganisms

Time interval (days)	Application rates mg Fosetyl-Al WG 80/kg dry weight soil				
	0	130.4		1304.0	
	Nitrate-N[1)]	Nitrate-N[1)]	% difference to control	Nitrate-N[1)]	% difference to control
0-7	-0.66±0.08	-0.75±0.01	13 n.s.	-0.43±0.04	35 *
7-14	0.52±0.18	0.53±0.10	1 n.s.	1.04±0.02	101 *
14-28	1.53±0.02	1.60±0.11	5 n.s.	2.20±0.09	43 *
28-42	1.59±0.05	1.46±0.03	8 n.s.*	1.24±0.14	22 **

1) Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation
* = Statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$).
n.s. = No statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$).
** = Statistically significant difference to the control (Welch-t Test for inhomogeneous variances, two-sided, $\alpha = 0.05$).
n.s.* = No statistically significant difference to the control (Welch-t Test for inhomogeneous variances, two-sided, $\alpha = 0.05$).

Observation:
During the 42-day test, 130.4 mg Fosetyl-Al WG 80 had no relevant influence on nitrogen transformation in a silty sand soil supplemented with Lucerne-grass-green meal. The 10-fold dose of the test item caused a temporary stimulation of the daily nitrate rates at the time intervals 0-7, 7-14 and 14-28 days after treatment. At the end of the experiment (28-42 day interval), differences in the nitrate-N rates between control soil samples and treated soil samples are < 25% and meet the trigger values of above mentioned guideline for a termination of the study.

Conclusions:
Fosetyl-Al WG 80 should not have an impact on nitrogen transformation in soils up to 1304 mg product/kg dwt soil.

Report: KCA 8.5/03 [redacted].; 2015; M-528580-01-1
Title: Dipotassium phosphonate (BCS-CZ96503): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.: 15 10 48 065 N
Document No.: M-528580-01-1
Guideline(s): OECD 216 (2000)
Guideline deviation(s): none
GLP/GEP: **yes**

Objective:
The purpose of this study was to determine the effects of dipotassium phosphonate (salt of phosphonic acid) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

Materials and Methods:
Test item: Dipotassium phosphonate (BCS-CZ96503), Batch Code.: BCS-CZ96503-PU-01, Origin Batch No.: SES 12947-1-1, LIMS No.: 1510480, CAS No.: 13492-26-7, Certificate No.: AZ20059, analysed purity: 98.5% w/w.

A silty sand soil (DIN 4220) was exposed for 42 days to 6.54 mg test item/kg soil dry weight and 65.31 mg test item/kg soil dry weight. Application rates were equivalent to 4.90 kg test item/ha and 48.98 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, 28 and 42 days after treatment).

Findings:

Validity Criteria of the Study

The coefficients of variation in the control (NO_3-N) were maximum 7.3% and thus fulfilled the demanded range (≤15%).

Effects on nitrogen transformation in soil after treatment with dipotassium phosphonate:

Time Interval (days)	Control			6.54 mg test item/kg soil dry weight equivalent to 4.90 kg test item/ha				65.31 mg test item/kg soil dry weight equivalent to 48.98 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.98	±	0.42	4.27	±	0.30	**+7.4** [n.s.]	4.94	±	0.57	**+24.2** [n.s.]
7-14	2.30	±	0.42	2.49	±	0.34	**+8.5** [n.s.]	1.73	±	0.42	**-24.7** [n.s.]
14-28	1.35	±	0.37	1.70	±	0.19	**+26.1** [n.s.]	1.90	±	0.14	**+40.4** [n.s.]
28-42	0.86	±	0.36	0.69	±	0.12	**-19.9** [n.s.]	0.71	±	0.08	**-17.2** [n.s.]

The 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 homogeneous variances, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused stimulations of the nitrogen transformation of +39.1%, +62.5% and +112.0% at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Observation:

The test item dipotassium phosphonate caused temporary stimulation of the daily nitrate rate at the tested concentrations of 6.54 mg test item/kg soil dry weight and 65.31 mg test item/kg soil dry weight at time interval 14-28 days after application.

However, no adverse effects of dipotassium phosphonate on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 42 days after application (time interval 28-42). Differences from the control of -19.9% (test concentration 6.54 mg test item/kg soil dry weight) and -17.2% (test concentration 65.31 mg test item/kg soil dry weight) were measured at the end of the 42-day incubation period (time interval 28-42).

Conclusions:

Dipotassium phosphonate (salt of phosphonic acid) caused no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 42-day incubation period. The study was performed in a field soil at concentrations up to 65.31 mg test item/kg soil dry weight, which are equivalent to application rates up to 48.98 kg test item/ha.

CA 8.6 Effects on terrestrial non-target higher plants

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. Studies have been conducted with the representative formulation Fosetyl-aluminium WG 80 and summaries can be found in Document MCP, Section 10.6.2.

CA 8.6.1 Summary of screening data

According to the data requirements for plant protection products (Commission Regulation No 284/2013), screening data shall be required for plant protection products other than those exhibiting herbicidal or plant growth regulator activity. However, for the representative formulations of the fungicide and bactericide fosetyl, guideline studies are available and are presented in the respective Document MCP, Section 10.6.2.

CA 8.6.2 Testing on non-target plants

Guideline studies have been conducted with the representative formulations, i.e. Fosetyl-aluminium WG 80 and Fosetyl-aluminium + Fluopicolide WG 71.11, and are presented in the respective Document MCP, Section 10.6.2.

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

In view of the study results presented above no studies on other terrestrial organisms are considered necessary.

CA 8.8 Effects on biological methods for sewage treatment

For information on studies already evaluated for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the corresponding section in the DAR and in the Baseline Dossier provided by Bayer CropScience. The study from which the endpoint will be used for risk assessment is summarised below from the original DAR of fosetyl.

Table 8.8- 1: Study on sewage treatment with fosetyl-Al

Test substance	Test species/ study type	Endpoint	References
Fosetyl-Al, tech.	Activated sludge, 3 h	EC_{50} > 100 mg a.s./L	[redacted].; 1999; M-179088-01-1 KCA 8.8/01

Report: KCA 8.8/01 [redacted].; 1999; M-179088-01-1
Title: Fosetyl-Al: Activated sludge - Respiration inhibition test.
Report No.: R009342
Document No.: M-179088-01-1
Guideline(s): EU (=EEC): Directive 88/302; OECD: 209
Guideline deviation(s): not specified
GLP/GEP: yes

Methods:
The study included the following groups: control (untreated, 2 replicates), test substance (1 replicate at 1 mg/L, 1 replicate at 10 mg/L and 3 replicates at 100 mg/L), and reference compound (3,5-dichlorophenol at 3.0, 10 and 32 mg/L). Purity of the test substance (978 g/kg).

Results:

EC_{50} – (3 h) > 100 mg/L

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary

Material and methods:
The effect of fosetyl-Al (Lot number OP 9950059, purity: 97.8%) on the respiration rate of activated sludge was assessed by the methods detailed in EC Directive 88/302, 'Biodegradation - Activated Sludge Respiration Inhibition test' and OECD Test Guideline 209, 'Activated Sludge, Respiration Inhibition test'.

Samples of activated sludge (suspended solids 1.6 g/L), fed with synthetic sewage were exposed to the test substance at nominal concentrations of 1, 10 and 100 mg/L for three hours. Single mixtures were prepared at 1 and 10 mg/L and the highest level was prepared in triplicate. Their rates of oxygen consumption were determined and compared with those of controls, containing activated sludge and synthetic sewage alone, which were established at the beginning and end of the culture series.

The reference inhibitor 3,5-dichlorophenol (3,5-DCP) was employed at 3.0, 10.0 and 32.0 mg/L as a positive control.

Findings:

Temperature, pH and measurements of respiration rate

Test mixture	Temp-erature (°C)		pH		Measured Dissolved Oxygen Concentration (mg O_2/L)		Respiration rate (mg O_2/g/h)	% inhibition ()*
	Initial	Final	Initial	Final	Initial	Final		
Control (1)	21.3	20.0	7.5	8.2	6.5	2.5	36.6	-
FOSETYL-AL (mg/L)								
1	21.7	20.1	7.5	8.0	6.0	2.5	39.8	0 (3)
10	21.7	20.0	7.5	8.0	6.0	2.5	42.3	0 (9)
100	21.3	20.0	6.8	7.9	6.5	2.5	42.9	0 (10)
100	21.3	20.0	6.8	7.9	6.5	2.5	42.9	0 (10)
100	21.1	20.2	6.9	7.9	6.5	2.5	42.9	0 (10)
3,5-DCP (mg/L)								
3.0	20.5	20.3	7.5	8.3	6.5	2.5	32.6	16
10.0	20.5	20.7	7.5	8.3	6.5	2.5	22.1	43
32.0	20.5	20.3	7.6	8.2	7.8	5.5	12.5	68
Control (2)	20.9	20.8	7.5	8.0	6.0	2.5	41.0	-

* Values given in parentheses refer to actual increases in respiration rate, expressed as a percentage of the mean control value (38.8 mg O_2/g/h).

Sludge respiration rates were progressively reduced in the presence of increasing concentrations of 3,5-DCP. The three hour 50% effect concentration (EC_{50}) for 3,5-DCP was calculated by the Moving Average method to be 14.0 mg/L (95% confidence limits 10.9 to 19.1 mg/L).

The specific respiration rate of the control culture established at the end of the test (41.0 mg O_2/g/h) was 112% of the rate of that established at the start (36.6 mg O_2/g/h).

These results show that the test was valid and that the sample of activated sludge employed was sensitive to inhibition. The three-hour EC_{50} for 3,5-DCP (14.0 mg/L) fulfilled the validity criterion relating to sensitivity to inhibition (acceptable EC_{50} range 5 to 30 mg/L), and that relating to the respiration rates in the control (variation not greater than 15%) was also satisfied.

Conclusions:
The criteria of validity specified in the test guideline were fulfilled in this study.
Fosetyl-Al had no inhibitory effect on the respiration rate of activated sludge at any of the concentrations employed in the test. The EC_{20}, EC_{50} and EC_{80} of the test substance could therefore not be calculated but these must be greater than 100 mg/L, the highest level tested.

CA 8.9 Monitoring data

No monitoring data have been collected by the applicant nor have they been reported in any of the public literature references as evaluated in Document MCA, Section 9. Due to the low to moderate acute and chronic ecotoxicity of fosetyl-aluminium as presented in Sections CA 8.3 to CA 8.9, no monitoring of non-target organism deem to be necessary.