Document Title

Summary of the ecotoxicological studies for Fosetyl-aluminium WG 80 (800 g/kg)

Data Requirements

EU Regulation 1107/2009 & EU Regulation 284/2013
Document MCP
Section 10: Ecotoxicological studies

According to the Guidance Document SANCO/10181/2013 for preparing dossiers for the approval of a chemical active substance

Date

2016-09-01

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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
2015-10-05	Original Document MCP – Section 10 of Supplementary Dossier	M-534835-02-1
2016-07-18	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 10. - 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 10. - Endpoints from study [redacted], J.S., 1988, M-162331-01-1, KCA 8.2.6.2/01, added to Table 10.2-1.	M-534835-03-1
2016-09-01	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 2016-07-27: - New risk assessments for aquatic organisms have been added to chapter CP 10.2.	M-534835-04-1

[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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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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-aluminium WG 80 (Fosetyl-Al WG 80), 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. Additional information requested by the RMS France on 2016-07-27 as follow up of the requests of 2016-04-04 and 2016-06-02 during the evaluation of the Supplementary Dossier is highlighted in green.

Fosetyl is the ISO common name for ethyl hydrogen phosphonate (IUPAC) but the aluminium salt fosetyl-aluminium (fosetyl-Al), a variant of fosetyl, is used in the formulated product.

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 behaviour.

The formulation Fosetyl-Al WG 80 is a water dispersible granule (WG) formulation containing 800 g/kg of fosetyl-Al. This formulation is registered throughout Europe on a wide range of crops under trade names such as Aliette. Fosetyl-Al WG 80 was already a representative formulation of BCS for the Annex I inclusion of fosetyl under Directive 91/414/EEC.

Use pattern considered in this risk assessment

Table 10- 1: Intended application pattern

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [kg prod./ha]	Maximum application rate, individual treatment (ranges) [kg a.s./ha] Fosetyl-Al
Orchards (Pome fruits)	BBCH 55-85	1-3	7-10	4.5	3.6

Definition of the residue for risk assessment

Justification for the residue definition for risk assessment is provided in Document MCA, Section 7.4.1.

Table10- 2: Definition of the residue for risk assessment

Compartment	Residue Definition
Soil	Fosetyl-Al, phosphonic acid
Surface water	Fosetyl-Al, phosphonic acid
Sediment	Phosphonic acid
Groundwater	Fosetyl-Al, phosphonic acid
Air	Fosetyl-Al

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment has been performed according to "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438), referred to in the following as "EFSA GD 2009".

CP 10.1.1 Effects on birds

Table 10.1.1- 1: Endpoints used in risk assessment

Test substance	Test design	Test species	Endpoint		Reference
Fosetyl-Al	acute toxicity	Bobwhite quail	LD_{50}	> 8000 mg a.s./kg bw [a)]	[redacted], N. L.; [redacted], C. N. K.; 1981; M-159690-01-1 KCA 8.1.1.1/01
	acute toxicity	Japanese quail	LD_{50}	4997 mg a.s./kg bw	[redacted], D. B.; [redacted], D. M.; [redacted], N. L.; 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], T. L.; [redacted], M. T.; 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** [c)]	
	dietary toxicity (short-term)	Bobwhite quail	LC_{50} LDD_{50}	> 20000 mg a.s./kg diet > 3122 mg a.s./kg bw/d	[redacted], N. L.; [redacted], C. N. K.; [redacted], R. H.; 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], N. L.; [redacted], C. N. K.; [redacted], R. H.; 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], S. P.; [redacted], M.; [redacted], J. B.; 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], R.; 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], S. M.; [redacted]. [redacted], J. B.; 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], S. J.; [redacted], S. M.; [redacted], J. B.; 1995; M-200041-01-1 KCA 8.1.1.2/03

Bold: endpoints used in risk assessment
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%.

Request from the RMS:

The calculation of an extrapolate LD50 value and the calculation of an LD50 based on the geometrical mean of the endpoints from several species are two methods indicated in the guidance document EFSA/2009/1438 for the determination of the relevant toxicity value for the acute TER estimation. However, the guidance document does not indicate if both methods could be combined. It is the RMS opinion that both methods should not be combined as the combination of these extrapolations would induce too much uncertainty in the obtained endpoint. The reliable toxicity value for acute is the LD50 of 4997 mg a.s./kg b.w.

Response from BCS:

According to the EFSA Guidance document, the geometric mean LD50 is a fully valid approach to assess the acute toxicity endpoint, appropriately maintaining the level of protection. In order to correctly calculate statistics like the geometric mean, unbound values should be avoided. Therefore it is necessary and appropriate to apply the very conservative extrapolation factor recommended in the EFSA GD to "LD50 > x" values before inclusion into the geomean. In the view of the notifier, it is therefore not to be expected that the combination of the two methods would unduly increase the uncertainty of the acute risk assessment estimate.

Request from the RMS:

In accordance with the guidance document EFSA (2009), a justification that no mortality or no clinical signs were observed during the test should be provided to exclude the dietary endpoint from the acute TER calculations. Please provide such justification.

Response from BCS:

In the Mallard duck short term dietary study with fosetyl-aluminium (fosetyl-Al), no mortalities or clinical signs were reported for the birds treated with fosetyl-Al. In the Bobwhite quail short term dietary study, no clinical signs were observed and a single mortality was observed among the birds receiving 20000 ppm of fosetyl-Al. However, this single mortality occurred on day 6 of testing, and at the same time one mortality also occurred in the untreated controls. Therefore it is questionable whether this single mortality at 20000 ppm is actually a treatment related effect, and with regard to the time course certainly not appropriate for the use in an acute risk assessment which addresses a single day of exposure.

Request from the RMS:
A justification that no risk assessment is required for the metabolite phosphonic acid would be suitable.

Response from BCS:
The toxicity of phosphonic acid, the major metabolite of fosetyl-Al, has been evaluated in birds. Due to the absence of notable toxicity of phosphonic acid (no mortalities or treatment related effects have been found up to the highest doses tested), a quantitative risk assessment is not considered necessary.

Table 10.1.1- 2: Toxicity data of the formulated product Fosetyl-Al WG 80

Test species	Test design	Endpoint	Reference
Bobwhite quail	acute, oral	LD_{50} > 6400 mg product/kg bw	[redacted] M: [redacted]; [redacted]; [redacted] 1999, M-184583-01-1 RCP 10.1.1.1/01

Table 10.1.1- 3: Relevant generic avian focal species for risk assessment on Tier 1 level according to EFSA GD (2009)

Crop scenario	Most critical window of relevance for generic focal species scenario	Generic focal species	Representative species	Short cut values for reproductive RA based on	
				RUD_{90}	RUD_m
Orchards 2 × 3.6 kg/ha BBCH 55-85 7d interval	Spring, Summer	Small insectivorous bird "tit"	Blue tit	46.8	18.2
	Crop directed application BBCH ≥ 40	Small insectivorous/worm feeding bird "thrush"	Robin	2.2	0.8
	Crop directed application BBCH ≥ 40	Small granivorous bird "finch"	Serin	8.2	3.8

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 4: Tier 1 acute risk assessment for birds

Crop scenario	Generic focal species	DDD			DDD	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Fosetyl-Al								
Orchards Spring, Summer	Small insectivorous bird "tit"	3×6	46.8	1.6	269.6	5039	19	10
Orchards BBCH ≥ 40	Small insectivorous/worm feeding bird "thrush"		2.2		12.7		398	
Orchards BBCH ≥ 40	Small granivorous bird "finch"		8.2		47.2		107	

The TER_A values calculated in the acute risk assessment on Tier 1 level exceed the a-priori-acceptability trigger of 10 for all evaluated scenarios. Thus, the acute risk to birds can be considered as low and acceptable without need for further, more realistic risk assessment.

Acute risk assessment for birds drinking contaminated water

In the EFSA GD (2009), section 5.5, step 1 the following guidance is given on the selection of relevant scenarios for assessing the risk of pesticides via drinking water to birds and mammals:

- Leaf scenario: Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation.
- Puddle scenario. Birds and mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil.

For the crops under assessment in this evaluation (orchards) the leaf scenario is not considered relevant. The risk for birds from drinking water in puddles is addressed in Table 10.1.1- 5.

Acute risk assessment for birds drinking contaminated water in puddles

Table 10.1.1- 5: Evaluation of potential concern for exposure of birds drinking water

Crop	K_{oc} [L/kg]	Application rate × MAF [g a.s./ha]	LD_{50} [mg a.s./ kg bw/d]	Ratio (Application rate × MAF) / LD_{50}	"Escape clause" No concern if ratio	Conclusion
Fosetyl-Al						
Orchards	0.1	3600 × 1.0 = 3600	5039	0.7	**≤ 50**	No concern

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.1- 6: Tier 1 reproductive risk assessment for birds

Crop	Generic focal species	DDD: Appl. rate [kg a.s./ha]	DDD: SV_m	DDD: MAF_m	DDD: f_{TWA}	DDD	NOAEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
Fosetyl-Al									
Orchards Spring, Summer	Small insectivorous bird "tit"	3.6	18.2	2.0	0.53	69.5	≥ 331	≥ **4.8**	5
Orchards BBCH ≥ 40	Small insectivorous/worm feeding bird "thrush"		0.8			3.1		≥ 108	
Orchards BBCH ≥ 40	Small granivorous bird "finch"		3.8			14.5		≥ 23	

Bold values do not meet the Tier 1 TER trigger

The TER_{LT} values calculated in the reproductive risk assessment on Tier 1 level do not exceed the a-priori-acceptability trigger of 5 for the small insectivorous bird scenario in orchards. Thus, a refined risk assessment for this scenario is presented below. The TER_{LT} values for the other scenarios exceed the a-priori-acceptability trigger of 5. Thus, no further risk assessment is needed for these scenarios.

Refined risk assessment – small insectivorous birds in orchards

Additional refinement potential can be employed by incorporating PT values for the blue tit in orchards as reported by Finch *et al.* (2006): mean PT = 0.21 for all birds (0.27 for "consumers"); 90th percentile PT = 0.55 for all birds (0.58 for "consumers);
A recalculation of the data already evaluated by Finch *et al.* (2006) has been provided in Prosser (2010): 90th percentile PT for blue tits in orchards: 0.53 for all birds (0.57 for consumers).

The documents with these PT values are accessible on the internet:

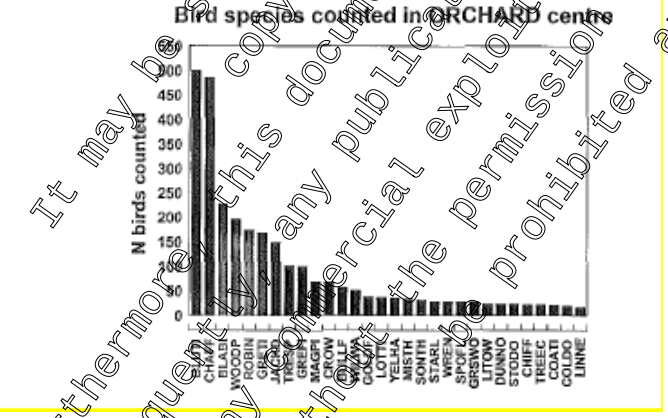
Finch *et al.*: 2006:
http://www.pesticides.gov.uk/Resources/CRD/Migrated-Resources/Documents/PPTFeb06.pdf

Prosser 2010:
http://randd.defra.gov.uk/Document.aspx?Document=10258_ConsolidationofbirdandmammalPTdataforuseinriskassessment.pdf

Request from the RMS:
For the refined reproduction risk assessment for birds, it would not be possible to validate that the blue tit is a relevant focal species for uses in orchard (pome fruits) without data. Indeed, without data the choice of blue tit as focal species for the refined risk assessment would be challenged during the peer-review. Could you please provide such data?

Response from BCS:
The proposed PT value for blue tits is taken from radiotracking work conducted by the former Central Science Laboratory. The selection of the species for radiotracking had been based on previous bird counting work conducted in UK orchards (reported 1998 as CONTRACT PN0903: IMPROVING THE ASSESSMENT OF PESTICIDE RISKS TO BIRDS IN ORCHARDS, Objective 2: Relative importance of pesticides and other factors influencing birds in orchards). Below a screenshot of the results as presented in the original CSL document on page 34. BLUTI is the acronym for the blue tit, the most abundant small insectivorous species in these surveys).

Fig 7a. The top 30 bird species recorded among orchard fruit trees
(See Appendix 5 for key to species names).



For illustration, below the screenshot of Table 3 on page 13 of Prosser 2010, providing highly conservative PT – value recommendations for blue tits in orchards.

Screenshot Table 3 on page 13 of Prosser 2010:

Table 3 PT values for passerine birds in orchards, with modelled 90th and 95th percentiles and their confidence limits. Consumers only.

Season	Species	No. of individuals	90th percentile PT value (95% CLs)	95th percentile PT value (95% CLs)
Summer (April – September)	Blackbird	28	0.73 (0.61 – 0.86)	0.83 (0.71 – 0.93)
	Blue tit	16	0.57 (0.43 – 0.75)	0.66 (0.52 – 0.84)
	Chaffinch	24	0.8 (0.69 – 0.91)	0.87 (0.77 – 0.96)
	Robin	24	0.54 (0.43 – 0.69)	0.65 (0.52 – 0.80)

The refined risk assessment calculation is provided in the table below.

Table 10.1.1- 7: Refined reproductive risk assessment for small insectivorous birds in orchards

Crop	Generic focal species	DDD: Appl. rate [kg a.s./ha]	DDD: SV_m	DDD: MAF_m	DDD: f_{TWA}	PT	DDD	NOAEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
Fosetyl-Al										
Orchards Spring, Summer	Small insectivorous bird "tit"	3.6	18.2	2.0	0.53	0.57	39.59	≥ 331	≥ 8.4	5

Uncertainty analysis

Refinement of the Tier 1 risk assessments is only triggered for one scenario in the reproductive risk assessment: small insectivorous birds ("tit") in orchards.

For this scenario, a single refinement element is introduced in the section above. Therefore it is considered appropriate and acceptable to focus the uncertainty analysis this element instead of a tabular approach as recommended in the EFSA GD (2009).

For the scenario of **small insectivorous birds ("tit") in orchards**, a 90th percentile PT value is available from radiotracking blue tits. This data is considered sufficient to address the long-term risk assessment

As all other elements of the exposure assessment remain unchanged in the refined risk assessment. The uncertainty in the sense of overlooking an undue risk for small insectivorous birds in orchards can be considered as low.

Therefore the long-term risk assessment for **small insectivorous birds ("tit") in orchards** is considered acceptable.

Long-term risk assessment for birds drinking contaminated water in puddles

Table 10.1.1- 8: Evaluation of potential concern for exposure of birds drinking water

Crop	K_{oc} [L/kg]	Application rate × MAF [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (Application rate × MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
Fosetyl-Al						
Orchards	0.1	3600 × 1.0 = 3600	≥ 331	≤ 11	**≤ 50**	No concern

RISK ASSESSMENT OF SECONDARY POISONING

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, an octanol-water partition coefficient ($\log P_{ow}$) > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation.

Table 10.1.1- 9: Log P_{ow} values of fosetyl-Al and its metabolite

Substance	log P_{ow}	Reference
Fosetyl-Al	- 2.1 (pH 6)	Document MCA, Section 2.7 EFSA Scientific Report 54 (2005)
Phosphonic acid - H_3PO_3	- 4.06 (pH 6)	Document MCA, Section 2.7

The log P_{ow} values of fosetyl-Al and phosphonic acid are below the trigger value of 3, indicating a very low risk of secondary poisoning.

CP 10.1.1.1 Acute oral toxicity

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

Report: KCP 10.1.1.1/01 [redacted]; 1999; M-184583-01-1

Title: EXP10369F: Acute oral toxicity (LD50) to bobwhite quail
Report No.: R01797
Document No.: M-184583-01-1
Guideline(s): USEPA (=EPA): E, 71-1, Equivalent to US EPA OPPTS Guideline No. 850.2100
Guideline deviation(s): not specified
GLP/GEP: **yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-79 for Fosetyl-Al WG 80:

LD_{50} > 6400 mg product/kg bw

Methods:
Test substance: Fosetyl-Al WG 80 (EXP10369F (794 g a.s./kg)). Each experimental group included 10 birds (5 males and 5 females). The three experimental groups were treated at nominal doses of 1600, 3200 and 6400 mg/kg b.w.

Results:
Two mortalities occurred during the study: one female in the treatment group at 6400 mg/kg on d 1 after dosing and one male in the 3200 mg/kg group on d 6. These two mortalities were considered likely to be related to treatment. Clinical signs of toxicity were observed in the bird which died on d 1. No clinical signs of toxicity were observed in any other bird. Food consumption was similar in treated and untreated groups. Bodyweight of males did not differ significantly between treated and untreated groups. Of female bodyweights in all treated groups were statistically lower than the controls on d 7. At the end of the test on d 14, there were no statistically significant differences in either males or females between the control and treated groups.

LD_{50} > 6 400 mg Fosetyl-Al WG 80/kg b.w.
NOEL: not determined

- **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 7	Day 14
Males			
Control	193	199	200
1600 mg/kg bw	189	193	195
3200 mg/kg bw	184	191	194
6400 mg/kg bw	190	192	197
Females			
Control	191	200	202
1600 mg/kg bw	190	194 *	198
3200 mg/kg bw	192	197 *	200
6400 mg/kg bw	188	191 **	195

Statistically significant differences: * p < 0.05 ** p < 0.01(

Mean food consumption [g/bird/d]:

Test group	Day -7 to -1	Day 1 to 7	Day 8 to 14
Males			
Control	13	14	13
1600 mg/kg bw	12	13	13
3200 mg/kg bw	12	12	14
6400 mg/kg bw	13	14	15
Females			
Control	12	13	13
1600 mg/kg bw	12	13	13
3200 mg/kg bw	13	14	15
6400 mg/kg bw	13	13	15

Summary table

Reference	Followed guidance	Guidance currently in force	Differences	Critical assessment of the study / Deviations / conclusion about its Reliability
M-184583-01-1 KCP 10.1.1.1/01	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 KCP 10.1.1.1/01 satisfies the requirements in OECD TG 223.

CP 10.1.1.2 Higher tier data on birds

In view of the results presented in Section CP 10.1.1.1, no further studies were necessary.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Table 10.1.2- 1: Endpoints used in risk assessment

Test substance	Exposure	Species	Endpoint	Reference
Fosetyl-Al	Acute risk assessment	Rat	LD_{50} > 7080 mg a.s./kg bw	[redacted], I.; 1997; M-179081-01-1 KCA 5.2.1/01
	Long-term risk assessment	Rat	NOAEL 6000 ppm ≡ 439 mg a.s./kg bw/d 6000 ppm NOAEL ≡ 720 mg/kg bw/d [a)]	[redacted] A.K.; [redacted] A.M.; [redacted] S.J.; [redacted], R.; [redacted] J.M.; [redacted] A.J.; [redacted] R.N.; [redacted] A.E.; 1981; M-203019-01-1 KCA 5.6.1/01

[a)] please refer to Document MCA, Section 8.1.2.2

Request from the RMS:
A justification that no risk assessment is required for the metabolite phosphonic acid would be suitable.

Response from BCS:
Phosphonic acid is a confirmed mammalian metabolite of fosetyl-aluminium (fosetyl-Al) and its toxicity was accounted for in the acute and long term studies with fosetyl-Al in mammals. Therefore, the mammalian risk assessment for fosetyl-Al also adequately addresses the risk for the phosphonic acid.

Table 10.1.2- 2: Relevant generic focal species for risk assessment on Tier 1 level acc. to EFSA GD (2009)

Crop scenario	Most critical window of relevance for generic focal species scenario	Generic focal species	Representative species	Short cut values for reproductive RA based on	
				RUD_{90}	RUD_m
Orchards 3 × 3.6 kg/ha BBCH 55-85 7d interval	BBCH ≥ 40	Small herbivorous mammal “vole”	Common vole	40.9	21.7
	Fruit stage BBCH 71-79 currants	Frugivorous mammal “dormouse”	Garden dormouse	47.9	22.7
	BBCH ≥ 40	Large herbivorous mammal “lagomorph”	Rabbit	10.5	4.3
	BBCH ≥ 40	Small omnivorous mammal “mouse”	Wood mouse	5.2	2.3

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 3: Tier 1 acute risk assessment for wild mammals

Crop	Generic focal species	DDD			DDD	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Fosetyl-Al								
Orchards BBCH ≥ 40	Small herbivorous mammal “vole”	3.6	40.9	1.6	235.6	> 7080	> 30	10
Orchards BBCH 71-79	Frugivorous mammal “dormouse”		47.9		275.9		> 26	
Orchards BBCH ≥ 40	Large herbivorous mammal “lagomorph”		10.5		60.5		> 117	
Orchards BBCH ≥ 40	Small omnivorous mammal “mouse”		5.2		30.0		> 236	

The TER_A values calculated in the acute risk assessment on Tier 1 level for wild mammals exceed the a-priori-acceptability trigger of 10 for all evaluated scenarios. Thus, the acute risk to wild mammals can be considered as low and acceptable without need for further, more realistic risk assessment.

Acute risk assessment for mammals drinking contaminated water
The puddle scenario is relevant for the acute risk assessment.

Table 10.1.2- 4: Evaluation of potential concern for exposure of mammals drinking water

Crop	K_{oc} [L/kg]	Application rate * MAF [g a.s./ha]	LD_{50} [mg a.s./kg bw/d]	Ratio (Application rate * MAF) / LD_{50}	“Escape clause” No concern if ratio	Conclusion
Fosetyl-Al						
Orchards	0.1	3600 x 1.0 = 3600	> 7080	< 0.5	**≤ 50**	No concern

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.2- 5: Tier 1 reproductive risk assessment for wild mammals

Crop	Generic focal species	DDD				DDD	NOAEL [mg a.s./kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Fosetyl-Al									
Orchards BBCH ≥ 40	Small herbivorous mammal "vole"	3.6	21.7	2.0	0.53	82.8	720	8.1	5
Orchards BBCH 71-79	Frugivorous mammal "dormouse"		22.7			86.6		8.3	
Orchards BBCH ≥ 40	Large herbivorous mammal "lagomorph"		4.3			16.4		44	
Orchards BBCH ≥ 40	Small omnivorous mammal "mouse"		2.3			8.8		82	

The TER_{LT} values calculated in the reproductive risk assessment on Tier 1 level for wild mammals exceed the a-priori-acceptability trigger of 5 for all evaluated scenarios. Thus, the long-term risk to wild mammals can be considered as low and acceptable without need for further, more realistic risk assessment.

Long-term risk assessment for mammals drinking contaminated water
The puddle scenario is relevant for the long-term risk assessment.

Table 10.1.2- 6: Evaluation of potential concern for exposure of mammals drinking water

Crop	K_{oc} [L/kg]	Application rate * MAF [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL)	"Escape clause" No concern if ratio	Conclusion
Fosetyl-Al						
Orchards	0.1	3600 x 1=3600	720	5.0	**≤ 50**	No concern

RISK ASSESSMENT OF SECONDARY POISONING

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log $P_{ow} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

As presented in Table 10.1.1- 9, log P_{ow} values are below the trigger value indicating a very low risk of secondary poisoning.

CP 10.1.2.1 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 corresponding section in the Baseline Dossier provided by Bayer CropScience and in the DAR.

Table 10.1.2.1- 1: Mammalian toxicity data of the formulated product Fosetyl-Al WG 80

Test species	Test design	Endpoint		Reference
Rat	acute, oral	LD_{50}	> 2000 mg product/kg bw	[redacted]B; 1999; M-199988-01-1 KCP 7.1.1/01

CP 10.1.2.2 Higher tier data on mammals

In view of the results presented above, no further studies were necessary. A number of assessments (M-237219-01-1, M-237426-01-1, M-236288-01-1, M-005688-01-1, M-237425-01-1) made for the Annex I inclusion of fosetyl under Directive 91/414/EEC and included in the original DAR and its Final Addendum are no valid or applicable any longer.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Information on effects of fosetyl on reptiles or amphibians is not available. No guidelines for studies with terrestrial amphibian life stages and reptiles are available and no risk assessment schemes are established so far. Therefore no further studies can be suggested for these groups of organisms.

CP 10.2 Effects on aquatic organisms

The risk assessment is based on the current guidance: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp.

Risk assessment for aquatic organisms

Ecotoxicological endpoints used in risk assessment

Table 10.2- 1: Endpoints used in risk assessment and additional studies for fosetyl-Al, its metabolite and Fosetyl-Al WG 80

Test substance	Test species	Endpoint		Reference
Fosetyl-Al WG 80	Fish, acute , *Oncorhynchus mykiss*	LC_{50}	> 120 mg product/L (nom) (> 96 mg a.s./L)	[redacted]; 1999; M-184613-01-1 KCP 10.2.1/01
	Invertebrate, acute *Daphnia magna*	EC_{50}	37 mg product/L (nom) (29.6 mg a.s./L)	[redacted],; 1999; M-184617-01-1 KCP 10.2.1/02
	Algae *Desmodesmus subspicatus* (*Scenedesmus subspicatus,* green algae*)*	E_bC_{50} E_rC_{50}	8.0 mg product/L (nom) (6.4 mg a.s./L) 27.7 mg product/L (nom) (22.2 mg a.s./L)	[redacted]; [redacted]; 1999; M-184628-01-1 KCP 10.2.1/03

Test substance	Test species	Endpoint		Reference
Fosetyl-Al	Fish, acute *Lepomis macrochirus*	LC_{50}	**> 60 mg a.s./L (mm)**	[redacted], P. M.; 1997; M-184497-01-1 KCA 8.2.2/02
	Fish, acute *Oncorhynchus mykiss*	LC_{50}	> 122 mg a.s./L (mm)	[redacted], G.; 1999; M-189219-01-1 KCA 8.2.1/01
	Fish, acute *Cyprinus carpio*	LC_{50}	> 100 mg a.s./L (nom)	[redacted], E.; 2013; M-449083-01-1 KCA 8.2.1/03
	Fish, chronic *Oncorhynchus mykiss*	NOEC	≥ 100 mg a.s./L (nom)	[redacted], P. M.; 1997; M-184573-01-1 KCA 8.2.2/01
	Fish, chronic *Pimephales promelas*	**NOEC**	**0.213 mg a.s./L (nom)**	[redacted], D.; [redacted], K.; 2015; M-531353-01-1 KCA 8.2.2/01
	Invertebrate, acute *Daphnia magna*	EC_{50}	**> 100 mg a.s./L (nom)**	[redacted], I. G.; 1996; M-176014-01-1 KCA 8.2.4.1/01
	Invertebrate, chronic *Daphnia magna*	**NOEC**	**17 mg a.s./L (nom)**	[redacted], I. G.; 1996; M-189214-01-1 KCA 8.2.5.1/01
	Algae *Desmodesmus subspicatus* (*Scenedesmus subspicatus*, green algae)	E_bC_{50} E_rC_{50}	8.9 mg a.s./L (mm) > 16 mg a.s./L (mm)	[redacted], G.; 1999; M-189220-01-1 KCA 8.2.6.1/01
	Algae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*, green algae)	7d-E_bC_{50} **72h-E_rC_{50}**	4.99 mg a.s./L (mm) recalculation **9.54 mg a.s./L (mm)**	[redacted], J. S.; 1989; M-163526-01-1 KCA 8.2.6.1/03 [redacted], M.; 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], M.; 2007; M-289324-01-1 KCA 8.2.6.1/05
	Algae *Navicula pelliculosa* (diatom)	7d-E_bC_{50} 72h-E_rC_{50}	8.93 mg a.s./L (mm) recalculation 18.11 mg a.s./L (mm)	[redacted], J.S.; 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], J. S.; 1989; M-163537-02-1 KCA 8.2.7/01 [redacted], C.; 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], J. W.; [redacted], H. J.; 1994; M-179069-01-1 KCA 8.2.1/03
	Fish, acute, *Oncorhynchus mykiss*	**LC_{50}**	**> 400 mg pm/L (nom)**	[redacted], M.; 2008; M-310496-01-1 KCA 8.2.1/06
	Fish, acute *Lepomis macrochirus*	LC_{50}	> 35.7 mg pm/L (nom) [b]	[redacted], M.; 1999; M-171840-01-1 KCA 8.2.1/04
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 29.7 mg pm/L (mm) [a]	[redacted], J. W.; [redacted], H. J.; 1994; M-179068-01-1 KCA 8.2.4.1/02
	Invertebrate, acute *Daphnia magna*	**EC_{50}**	**> 400 mg pm/L (nom)**	[redacted], M.; 2008; M-310318-01-1 KCA 8.2.4.1/03
	Sediment dweller *Chironomus riparius*	**NOEC**	**≥ 100.2 mg pm/L (nom)** [b]	[redacted], M.; 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], M. J.; [redacted], M.; [redacted], D.; 1999; M-171844-01-1 KCA 8.2.6.1/02

Bold: endpoints used in risk assessment
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%.

Selection of algae and macrophytes endpoints for risk assessment

Processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) is more suitable to measure effects in algae and macrophytes. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for yield or biomass based endpoints. Following current state of science, the test guidelines OECD TG 201 and 221, the EU-Method C3, the EC regulation for Classification and Labeling (EC regulation 1272/2008), the PPR Opinion (EFSA Journal 461, 1-44; 2007) and also the EFSA Aquatic Guidance Document (AGD, 2013, noted by SCFCAH on July 10-11th, 2014), list growth rate as the relevant endpoint of the algae and the *Lemna* growth inhibition test. The previous Guidance Document on Aquatic Toxicology (SANCO/3268/2001 rev. 4) still stated that "As there is no clear evidence available to indicate which is the most relevant endpoint for the field situation, the lower figure should be used in the risk assessment". As this statement is clearly superseded by recent

scientific and regulatory developments toxicity-exposure-ratios in this assessment were based on the E_rC_{50}, when available.

Predicted environmental concentrations used in risk assessment

Table 10.2- 2: Initial max PECsw values – FOCUS Steps 1 and 2

Compound	FOCUS Scenario	Orchards 3 × 3.6 kg a.s./ha, 7 d int., BBCH 55-85	new PEC_{sw} according to RMS request: Orchards 3 × 3.6 kg a.s./ha, 7 days int., BBCH 55-85
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Fosetyl-Al	STEP 1	4166.0	4166.0
	STEP 2 – North [A]	188.7	188.7
	STEP 2 - South [A]	188.7	188.7
Phosphonic acid	STEP 1	2842.0	3905.5
	STEP 2 – North [A]	229.2	329.2
	STEP 2 - South [A]	307.6	445.4

[A] Worst case values for single or multiple application

Table 10.2- 3: Initial max PECsw values – FOCUS Step 3

Compound	FOCUS Scenario	Orchards 3 × 3.6 kg a.s./ha, 7 d int., BBCH 55-85
		$PEC_{sw, max}$ [A] [µg/L]
Fosetyl-Al	D3 (ditch, 1st)	132.10
	D4 (pond, 1st)	5.929
	D4 (stream, 1st)	132.60
	D5 (pond, 1st)	5.930
	D5 (stream, 1st)	143.20
	R1 (pond, 1st)	5.925
	R1 (stream, 1st)	99.71
	R2 (stream, 1st)	136.20
	R3 (stream, 1st)	143.20
	R4 (stream, 1st)	99.32

[A] Worst case values for single or multiple application

Table 10.2- 4: Summary of FOCUS Step 4 PEC_{sw} values of fosetyl-Al (3×3.6 kg a.s./ha, 7d int.) Entries marked with * result from single applications. Pome/stone fruit, late applications

Fosetyl-Al [µg/L]													
Buffer Width & Type	Scenario	Nozzle Reduction											
		0%			50%			75%			90%		
5m Spray drift	D3 (Ditch)	S	89.130	*	S	44.570	*	S	22.280	*	S	8.9130	*
	D4 (Pond)	S	6.7810	*	S	3.3900	*	S	1.6950	*	S	0.6781	*
	D4 (Stream)	S	103.50	*	S	51.750	*	S	25.870	*	S	10.350	*
	D5 (Pond)	S	6.7820	*	S	3.3910	*	S	1.6960	*	S	0.6782	*
	D5 (Stream)	S	111.80	*	S	55.890	*	S	27.940	*	S	11.180	*
	R1 (Pond)	S	6.7760	*	S	3.3880	*	S	1.6940	*	S	0.6776	*
	R1 (Stream)	S	77.830	*	S	38.910	*	S	19.460	*	S	7.7830	*
	R2 (Stream)	S	106.30	*	S	53.150	*	S	26.580	*	S	10.630	*
	R3 (Stream)	S	111.80	*	S	55.890	*	S	27.950	*	S	11.180	*
	R4 (Stream)	S	77.520	*	S	38.760	*	S	19.380	*	S	7.7520	*
10m Spray drift & Runoff	D3 (Ditch)	S	39.830	*	S	19.920	*	S	9.9580	*	S	3.9830	*
	D4 (Pond)	S	3.7610	*	S	1.8800	*	S	0.9402	*	S	0.3761	*
	D4 (Stream)	S	46.250	*	S	23.120	*	S	11.560	*	S	4.6250	*
	D5 (Pond)	S	3.7620	*	S	1.8810	*	S	0.9404	*	S	0.3762	*
	D5 (Stream)	S	49.950	*	S	24.970	*	S	12.490	*	S	4.9950	*
	R1 (Pond)	S	3.7580	*	S	1.8790	*	S	0.9395	*	S	0.3758	*
	R1 (Stream)	S	34.780	*	S	17.390	*	S	8.6940	*	S	3.4780	*
	R2 (Stream)	S	47.500	*	S	23.750	*	S	11.880	*	S	4.7500	*
	R3 (Stream)	S	49.950	*	S	24.980	*	S	12.490	*	S	4.9950	*
	R4 (Stream)	S	34.640	*	S	17.320	*	S	8.6600	*	S	3.4640	*
15m Spray drift & Runoff	D3 (Ditch)	S	20.110	*	S	10.060	*	S	5.0280	*	S	2.0110	*
	D4 (Pond)	S	2.4030	*	S	1.2010	*	S	0.6007	*	S	0.2403	*
	D4 (Stream)	S	23.350	*	S	11.680	*	S	5.8380	*	S	2.3350	*
	D5 (Pond)	S	2.4030	*	S	1.2020	*	S	0.6008	*	S	0.2403	*
	D5 (Stream)	S	25.220	*	S	12.610	*	S	6.3050	*	S	2.5220	*
	R1 (Pond)	S	2.4010	*	S	1.2010	*	S	0.6003	*	S	0.2401	*
	R1 (Stream)	S	17.560	*	S	8.7800	*	S	4.3900	*	S	1.7560	*
	R2 (Stream)	S	23.980	*	S	11.990	*	S	5.9960	*	S	2.3980	*
	R3 (Stream)	S	25.220	*	S	12.610	*	S	6.3050	*	S	2.5220	*
	R4 (Stream)	S	17.490	*	S	8.7450	*	S	4.3730	*	S	1.7490	*
20m Spray drift & Runoff	D3 (Ditch)	S	12.290	*	S	6.1450	*	S	3.0730	*	S	1.2290	*
	D4 (Pond)	S	1.7110	*	S	0.8555	*	S	0.4277	*	S	0.1711	*
	D4 (Stream)	S	14.270	*	S	7.1360	*	S	3.5680	*	S	1.4270	*
	D5 (Pond)	S	1.7110	*	S	0.8556	*	S	0.4278	*	S	0.1711	*
	D5 (Stream)	S	15.410	*	S	7.7070	*	S	3.8530	*	S	1.5410	*
	R1 (Pond)	S	1.7100	*	S	0.8549	*	S	0.4274	*	S	0.1710	*
	R1 (Stream)	S	10.730	*	S	5.3660	*	S	2.6830	*	S	1.0730	*
	R2 (Stream)	S	14.660	*	S	7.3290	*	S	3.6650	*	S	1.4660	*
	R3 (Stream)	S	15.410	*	S	7.7070	*	S	3.8540	*	S	1.5410	*
	R4 (Stream)	S	10.690	*	S	5.3450	*	S	2.6720	*	S	1.0690	*

S, R and D denote main entry route via spray drift, runoff or drainage, respectively

Risk assessment for aquatic organisms

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 5: TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Orchards						
Fosetyl-Al	Fish, acute	LC_{50}	> 60000	188.7	> 318	100
	Invertebrate, acute	EC_{50}	> 100000		> 530	
Phosphonic acid	Fish, acute	LC_{50}	> 400000	307.6	> 1300	
	Invertebrate, acute	EC_{50}	> 400000		> 1300	

As requested by the RMS France, new PEC_{sw} calculations were performed using the input parameters as provided by ANSES. As the PEC_{sw} values for fosetyl-Al did not change due to the new calculations the risk assessment for fosetyl-Al remains unchanged. In the following an updated risk assessment is presented for the metabolite phosphonic acid, based on new maximum FOCUS Step 2 PEC_{sw} values for orchards.

Table 10.2- 5a: TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Orchards						
Phosphonic acid	Fish, acute	LC_{50}	> 400000	445.4	> 898	100
	Invertebrate, acute	EC_{50}	> 400000		> 898	

Request from the RMS:
The preparation seems to be more toxic than fosetyl-Al. The toxicity data of the preparation should also be used in the risk assessment (TER estimation for the preparation based on PECsw estimated for the drift of a single application is required).

Response from BCS:
The formulated product was shown to be of low/moderate toxicity to all 3 taxonomic groups of aquatic organisms (fish, *Daphnia* and green algae), and this is consistent with the toxicity of the active substance. Although there is no substantial difference between the toxicity of Fosetyl-Al WG 80, compared to the active substance, for fish and green algae, a difference of up to a factor of 3 can be estimated for *Daphnia*. However, such a factor is deemed to be within the biological variation inherent in standard laboratory studies. As such, these data do not indicate a real difference in toxicity between the formulated product and the active substance. Thus, the risk assessment performed below on the active substance fosetyl-aluminium (fosetyl-Al) will also cover the formulated product, and this is confirmed by TER_A calculations for the formulated product (see table below).

TER_A calculations based on FOCUS Step 2 for the representative formulation Fosetyl-Al WG 80

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Orchards						
Fosetyl-Al WG 80	Fish, acute	LC_{50}	> 120000	188.7	> 636	100
	Invertebrate, acute	EC_{50}	> 37000		> 196	
Fosetyl-Al a.s.	Fish, acute	LC_{50}	> 96000	188.7	> 509	
	Invertebrate, acute	EC_{50}	> 296000		> 1569	

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 6: TER$_{LT}$ calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		PEC$_{sw.max}$ [µg/L]	TER$_{LT}$	Trigger
Orchards						
Fosetyl-Al	Fish, chronic	NOEC	213	188.7	**1.1**	10
	Invertebrate, chronic	NOEC	17000		90.1	
	Green algae, chronic	E_rC_{50}	9540		50.6	
	Aquatic plants, chronic	E_rC_{50}	166600		883	
Phosphonic acid	Sediment dweller, chronic	NOEC	> 100200	307.6	> 326	
	Green algae, chronic	E_rC_{50}	29400		95.6	

Bold values do not pass the risk assessment

As requested by the RMS France, new PEC$_{sw}$ calculations were performed using the input parameters as provided by ANSES. As the PEC$_{sw}$ values for fosetyl-Al did not change due to the new calculations the risk assessment for fosetyl-Al remains unchanged. In the following an updated risk assessment is presented for the metabolite phosphonic acid, based on new maximum FOCUS Step 2 PEC$_{sw}$ values for orchards.

Table 10.2- 6a: TER$_{LT}$ calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		PEC$_{sw.max}$ [µg/L]	TER$_{LT}$	Trigger
Orchards						
Phosphonic acid	Sediment dweller, chronic	NOEC	> 100200	445.4	> 225	10
	Green algae, chronic	E_rC_{50}	29400		66	

Request from the RMS:
The chronic risk assessment for *Chironomus riparius* (phosphonic acid) should be done with the toxicity endpoint and the PECsed 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 to the sediment dweller *Chironomus riparius* is derived from the study by [redacted]; 1999; M-171912-01-1 (please refer to Document MCA, Section 8.2.5.4, KCA 8.2.5.4/01), 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 days 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), thus as mg/L.

All TER values for the uses in orchards meet the trigger value based on FOCUS Step 2 PEC_{sw} values, except for the long-term exposure to fish. Therefore TER calculations for fish based on FOCUS Step 3 values are presented below.

Table 10.2- 7: Refined TER calculations for fosetyl-Al based on FOCUS Step 3

Compound	Species	Endpoint [µg/L]	FOCUS scenario	$PEC_{sw,max}$ [µg/L]	TER	Trigger
Orchards						
Fosetyl-Al	Fish, chronic	NOEC 213	D3 (ditch, 1st)	132.10	**1.6**	10
			D4 (pond, 1st)	5.929	35.9	
			D4 (stream, 1st)	132.60	**1.6**	
			D5 (pond, 1st)	5.930	35.9	
			D5 (stream, 1st)	143.20	**1.5**	
			R1 (pond, 1st)	5.925	35.9	
			R1 (stream, 1st)	99.71	**2.1**	
			R2 (stream, 1st)	136.20	**1.6**	
			R3 (stream, 1st)	143.20	**1.5**	
			R4 (stream, 1st)	99.32	**2.1**	

Bold values do not pass the risk assessment

The FOCUS pond scenarios meet the required trigger. Nevertheless, further refinement using FOCUS Step 4 values is necessary for all stream and ditch scenarios and is presented below.

Table 10.2- 8: Refined TER calculations for fosetyl-Al based on FOCUS Step 4 including mitigation measures

Species	Endpoint [µg/L]	Mitigation	FOCUS scenario	$PEC_{sw,max}$ [µg/L]	TER	Trigger
Orchards						
Fish, chronic	NOEC 213	20 m vegetated buffer strip	D3 (ditch)	12.29	17.3	10
			D4 (stream)	14.27	14.9	
			D5 (stream)	15.41	13.8	
			R1 (stream)	10.73	19.9	
			R2 (stream)	14.66	14.5	
			R3 (stream)	15.41	13.8	
			R4 (stream)	10.69	19.9	
Fish, chronic	NOEC 213	5 m non-spray buffer zone + 90% reducing nozzles	D3 (ditch)	8.913	23.9	10
			D4 (stream)	10.35	20.6	
			D5 (stream)	11.18	19.1	
			R1 (stream)	7.783	27.4	
			R2 (stream)	10.63	20.0	
			R3 (stream)	11.18	19.1	
			R4 (stream)	7.752	27.5	
Fish, chronic	NOEC 213	10 m vegetated buffer strip + 75% reducing nozzles	D3 (ditch)	9.958	21.4	10
			D4 (stream)	11.56	18.4	
			D5 (stream)	12.49	17.1	
			R1 (stream)	8.694	24.5	
			R2 (stream)	11.88	17.9	
			R3 (stream)	12.49	17.1	
			R4 (stream)	8.660	24.6	

According to the presented risk assessment based on FOCUS Step 4 calculations, the risk to aquatic organisms from the use of the product in orchards is unlikely if
- 5 m non-spray buffer zone and 90% drift reduction, or
- 10 m vegetated buffer strip and 75% drift reduction, or
- 20 m vegetated buffer strip

are maintained during application of the product.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and 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 summaries from the DAR are given below.

Report:	KCP 10.2.1/01[redacted]; 1999; M-184613-01-1
Title:	EXP10369F: Acute toxicity for rainbow trout (*Oncorhynchus mykiss*)
Report No.:	R011807
Document No.:	M-184613-01-1
Guideline(s):	EU (=EEC): 92/69/EEC, C1 OECD: 203, Equivalent to US EPA OPPTS Guideline No. 850.1075
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 64, 1-99 for fosetyl-Al:

LC_{50} - 96 h > 120 mg fosetyl-Al WG 80/L (96 mg a.s./L)

Methods:
The test substance was EXP10369F (794 g fosetyl-Al/kg). A total of 40 juvenile fish from the same batch were used. The experimental design 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 EXP10369F (120 mg/L) equivalent to a nominal concentration of 95 mg fosetyl-Al/L). The test (limit test) was performed under semi-static conditions (daily renewal).

Results:
The test substance was soluble in the dilution water at the concentrations tested. Measured concentrations ranged from 103 to 104% of nominal values at both 0 and 24 h, from 102 to 103% at 72 h and from 100 to 104% at 96 h. The toxicity values are expressed in terms of nominal concentration of the formulated product. No mortality or sublethal toxicity was observed in the control groups or in the two groups of rainbow trout exposed for 96 h to a nominal concentration of 120 mg EXP10369F/L.

LC_{50} - 96 h > 120 mg EXP10369F/L (mean measured concentrations)
NOEC - 96 h = 120 mg EXP10369F/L (mean measured concentrations)

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary

Objective:
This study was designed to assess the acute toxicity of EXP 10369F (794 g fosetyl-Al/kg) to rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions.

Materials and methods:
Test item: EXP10369F, Lot No.: OP980953.
Bioassay was conducted over a period of 96 hours with the rainbow trout (*Oncorhynchus* mykiss) in filtered, dechlorinated and softened laboratory tap water. Chlorine levels ranged from 0.02 to 0.09 mg/L and the hardness level was between 132 and 168 mg $CaCO_3$/L. Medium was renewed daily.

A representative sample of stock fish was measured at the start of the study. The mean standard length was 3.8 cm (SD = 0.2 cm) and the mean weight was 0.86 g (SD = 0.2 g) resulting in a loading of 0.43 g bodyweight/L. Fish were not fed during exposure period.
A photoperiod of 16 hours light : 8 hours dark was maintained, and temperature, pH and dissolved oxygen were recorded daily in each control and test vessel.
Concentrations of the test substance were measured at 0 and 72 hours in fresh media and at 24 and 96 hours in expired media by ion chromatography using a conductivity detector and a suppressor.
Observations for any mortalities or incidence of sublethal effects in the fish compared to control were made at approximately 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 within 13 to 14 °C, *i.e.*, in the recommended range of 13 to 17 °C.

Analytical findings:
The pH values in the test ranged from 7.2 to 8.1, dissolved oxygen concentrations ranged from 8.1 to 10.1 mg/L, and temperature ranged from 13 to 14 °C.

Conclusion
The acute toxicity of EXP10369F to *Oncorhynchus mykiss* has been investigated and gave the 96-hour LC_{50} of > 120 mg EXP10369F/L (based upon nominal concentrations). The no observed effect concentration (NOEC) was 120 mg EXP10369F/L based on the lack of mortality and sublethal effects at this test concentration.

Report: KCP 10.2.1/02 [redacted]; 1999; M-184617-01-1
Title: EXP10369F: Acute toxicity to *Daphnia magna*
Report No.: R011802
Document No.: M-184617-01-1
Guideline(s): EU: EEC: 92/69/EEC, C2; OECD: 202, Part I;
Equivalent to US EPA OPPTS Guideline No. 850.1010
Guideline deviation(s): Not specified
GLP/GEP: yes

Endpoint according to EFSA Scientific Report (2005) 84, 1-79 for fosetyl-Al:
EC_{50} - 48h = 37 mg Fosetyl-Al WG 80/L (29.6 mg a.s./L)

Methods:
Daphnids less than 24-hold were distributed into the test vessels randomly and assigned to eight experimental groups (5 animals per replicate, 4 replicates per group). The experimental groups were a dilution water control, and 7 nominal concentrations (5.6, 10, 18, 32, 56, 100 and 180 mg/L) of EXP10369F (794 g fosetyl-Al/kg), equivalent to nominal concentrations of fosetyl-Al of 4.5, 7.9, 14, 25, 44, 79 and 142 mg a.s./L. The total duration of the period of exposure was 48 h.

Results:
Analytical verification showed that the measured concentrations of fosetyl-Al were close to nominal values. Measured concentrations ranged from 101 to 107% of nominal values at start and from 97 to 103% at the end of the test.

EC_{50} - 48 h = 37 mg EXP10369F/L (nominal concentration) (29.6 mg a.s./L)
NOEC - 48 h = 5.6 mg EXP10369F/L (nominal concentration) (4.5 mg a.s./L)

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary

Objective:
This study was undertaken to determine the acute toxicity, expressed as the 48-h median effect concentration (EC_{50}), of EXP 10369F (794 g fosetyl-Al/kg) to *Daphnia magna*.

Materials and methods:
Test item: EXP10369F, Lot No.: OP980953, purity: 794 fosetyl-Al/kg.
Bioassay was conducted with Elendt 4 medium without renewal with a photoperiod of 16 hours light : 8 hours dark and without supplementary aeration or feeding during the 48 hour exposure period. Each test vessel contained 5 daphnids and 100 mL of test solution to give a loading of 20 mL test solution per organism. The temperature, pH and dissolved oxygen levels were recorded at the start and at the end of the study. Test concentrations were verified by chemical analysis at 0 and 48 hours (LOD = 2 mg/L)

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 ≥ 3mg/L)	≥ 4.9 mg/L

Analytical findings:

Nominal concentration (mg/l)	**0 hours**			**48 hours[1]**		
	pH	**mg O_2/L**	**T °C**	**pH**	**mg O_2/L**	**T °C**
Control	7.9	7.0	21	7.6 - 7.7	7.2 – 7.3	22
5.6	7.7	7.2	21	7.7	7.3	22
10	7.6	7.0	21	7.7	7.2 – 7.5	22
18	7.5	7.1	21	7.7	7.2	22
32	7.4	7.2	21	7.6	7.2	22
56	7.2	6.8	21	7.5 - 7.6	7.2 – 7.4	22
100	7.0	7.1	21	7.3 - 7.4	7.2 – 7.5	22
180	5.2	6.9	21	4.9 - 5.0	7.3	22

[1] conducted with 4 replicates

Biolocical findings:
Number of immobilised *Daphnia* is displayed in table below.

Cumulative immobilisation data for *Daphnia magna* exposed for 48 hours to EXP10369F

Nominal concentration (mg/l)	24 hours		48 hours	
	Total	%	Total	%
Control	0	0	0	0
5.6	0	0	0	0
10	0	0	3	15
18	0	0	10	50
32	0	0	8	40
56	0	0	13	65
100	1	5	12	60
180	20	100	20	100

Conclusion
The acute toxicity study of EXP10369F to *Daphnia magna* has been investigated and gave a 48-hour EC_{50} of 37 mg EXP10369F/L (nominal concentration, 29.6 mg a.s./L). Accordingly, the NOEC was 5.6 mg EXP10369F/L (nominal concentration, 4.5 mg a.s./L).

Report: KCP 10.2.1/03[redacted]; 1999; M-184628-01-1
Title: EXP10369F: Algal growth inhibition assay on *Scenedesmus subspicatus*
Report No.: R011813
Document No.: M-184628-01-1
Guideline(s): EU (=EEC): 92/69/EEC, C3; OECD: 201, (1984);
Equivalent to US EPA OPPTS Guideline No 850.5400
Guideline deviation(s): Algal inoculum was cultured using a shaker on a light bench in a temperature controlled room, not in an incubator as stated in the protocol. This was because large volumes of inoculum were required.
This deviation did not affect the outcome of the study.
GLP/GEP: **yes**

Endpoint according to EFSA Scientific Report (2015) 54, 1-79 for fosetyl-Al:
E_rC_{50} - 72 h = 27.7 mg Fosetyl-Al WG 80/L (22.2 mg a.s./L)

Methods:
Nominal concentrations: 2.4, 5.2, 11.4, 25, 55, and 120 mg/L of EXP10369F (794 g fosetyl-Al/kg), equivalent to 1.9, 4.1, 9.1, 20, 44 and 95 mg a.s./L. The test substance was soluble in the test solution at all the concentrations tested.

Results:
Measured concentrations ranged from 79 to 96% of nominal values at 0 h and from 84 to 112% of nominal at 72 h. The results of this test are expressed in terms of the nominal concentrations of the test substance.

E_rC_{50} - 72 h = 27.7 mg EXP10369F/L
E_{50} - 72 h = 8.0 mg EXP10369F/L (C.I. 95%: 6.64 – 9.68)
NOEC - 72 h = 2.4 mg EXP10369F/L

- **Comments (RMS):** acceptable

Further study information supplementing the original Monograph summary

Objective:
This study was designed to assess the inhibitory effect of EXP 10369F (794 g fosetyl-Al/kg) on the growth of the unicellular alga *Scenedesmus subspicatus*.

Materials and methods:
Study was conducted using the strain No. CCAP 276/20 of the unicellular green algae *Scenedesmus subspicatus*. Triplicate algal cultures were exposed to six test levels with six replicates of an untreated control. Cultures were incubated in a Gallenkamp Orbital Incubator under continuous illumination of approximately 6000 lux at 22 °C for 72 hours. Culture medium was a sterile nutrient medium as recommended. The starting culture cell density was 5×10^4 cells/ml.
Test concentrations were verified by chemical analysis (LOD = 0.04 mg a.s./L, LOQ = 1.2 mg formulation/L). Duplicate samples were taken from control and test cultures at 0 and 72 hours.
The temperature and pH of the test cultures was measured at 0 and 72 hours.
Measurements of growth were conducted at 24, 48 and 72 hours and cell density was determined by using a haemocytometer. The areas under the growth curve (biomass) and average growth rate were calculated.

Findings:

Validity criteria:

Validity criteria (according to OECD 201, adopted 23.03.2006)	**Obtained in this study**
	control
Minimum increase in biomass during exposure period (criterion is by a factor of 16)	> 16
Mean coefficient of variation for section by section specific growth rates (days 0-1, 1-2, 2-3) in the controls (criterion is ≤ 35%)	72.3% (see argumentation below)
Coefficient of variation for average specific growth rates during the 0 to 72 hour test period in replicate control cultures (criterion is ≤ 10%)	≤ 10%

Biological results:

Nominal concentration (mg/l)	**Area under curve at 72 hours**	**% Inhibition**	**Growth rate (0 – 72 hours)**	**% Inhibition**
Control	38	-	0.052	-
2.4	40	<5.5>	0.054	<3.3>
5.2	23	38	0.048	8.1
11.4	13	66	0.040	23
25	6.2	84	0.032	39
55	-0.04	100	0.013	74
120	-2.7	107	0.019	136

Conclusion
Under laboratory conditions, EXP 10369F had an effect on the biomass and the growth rate of *Scenedesmus subspicatus*. The 72 hour E_bC_{50} was calculated to be 8.0 mg/L and the 72 hour E_rC_{50} was calculated to be 28 mg/L. The NOEC (no observed effect concentration) was calculated to be 2.4 mg/L.

Request from the RMS:
Further explanations are required to justify that the study of the effects of the preparation on *Scenedesmus subspicatus* ([redacted].,;[redacted]; 1999; M-184628-01-1) are still reliable for the risk since the coefficient of variation is estimated to be 72% for the control.

Response from BCS:
Whereas the biomass increases and the coefficient of variation of average specific growth rates in replicate controls over 72 hours meet the validity criteria according to the OECD TG 201, the RMS is right when stating that the coefficient of variation for section-by-section specific growth rates of controls is 72% (BCS´s calculations result in the value of 72.3%). BCS therefore concurs with the RMS that this very criteria is not met. Visual inspection of the growth curves (Figure 1 in the study report) shows that this is due to the slower growth of control algae over the first 24 hours. However, over this early time period, the pattern of toxic effects was already set, with a concentration-response relationship fully consistent with the final result of the study. Thus, highly variable section-by-section specific growth rates in the control had no major influence on the derivation of reliable toxicity endpoints from this study

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No new studies were necessary based on the current data requirements. Please refer to Document MCA, Section 8.2.

CP 10.2.3 Further testing on aquatic organisms

No studies were necessary based on the current data requirements. Please refer to Document MCA, Section 8.2.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The risk assessment has been performed according to the existing guidance in force at the time of the preparation and submission of this dossier namely the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/ 10329/2002 rev 2) and EPPO Standard PP 3/10 (3) Environmental Risk Assessment Scheme for Plant Protection Products - Chapter 10: honey bees.

Commission Regulations (EU) 283/2013 and 284/2013 require, where bees are likely to be exposed, testing by both acute (oral and contact) and chronic toxicity, including sub-lethal effects, to be conducted. Consequently in addition to the standard toxicity studies performed with adult bees (OECD 213 and 214) the following additional studies are also provided:

- 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),

- Semi-field studies following OEPP/EPPO Guideline No. 170(4) simulating a spray exposure scenario for honey bees in flowering apple orchards at the maximum application rate for the approval renewal of fosetyl and evaluating flight intensity, mortality and colony development.

Details of the bee testing with fosetyl-Al and ecotoxicological endpoints are presented in Document MCA, Section 8.3.1, Document MCP, Section 10.3.1, as well as within the existing EFSA Scientific Report (2005) 54, 1-79.

Table 10.3.1- 1: EU evaluated and additional studies on bee toxicity of fosetyl-Al, phosphonic acid and Fosetyl-Al WG 80

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], S.; [redacted], H.; 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 42 µg a.s./bee LD_{50} – contact > 1000 µg a.s./bee	[redacted], [redacted], [redacted], M.; 1999; M-092174-01-1 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], S.; 2012; M-440802-01-1 KCA 8.3.1.1.1/04 KCA 8.3.1.1.2/04
Fosetyl-Al WG 80	Honey bee, 48 h	LD_{50} – oral >440 µg prod/bee >352 µg a.s./bee LD_{50} – contact >390 µg prod/bee >310 µg a.s./bee	[redacted],; 1999; M-184602-01-1 KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
	Honey bee, 48 h	LD_{50} – oral **>136.3 µg prod/bee** **>110.4 µg a.s./bee** LD_{50} – contact **>123.5 µg prod/bee** **>100.0 µg a.s./bee**	[redacted],; 2014; M-503644-01-1 KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02
Phosphonic acid	Honey bee, 48 h	LD_{50} – oral > 212 µg p.m./bee	[redacted], S.; 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], S. J.; [redacted], J. B.; 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], T.; 2010; M-389965-01-1 KCA 8.3.1.1.1/05 KCA 8.3.1.1.2/05

Test substance	Test species/ study type	Endpoint	References
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], A.; 2015; M-527665-01-1 KCA 8.3.1.2/01
	Honey bee brood feeding (Oomen *et al.*, 1992)	Slightly 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], C.; 2015; M-508986-01-2 KCA 8.3.1.3/01
	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], B.; 2015; M-526896-01-1 KCA 8.3.1.3/02
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, 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], B.; 2015; M-528899-01-1 KCA 8.3.1.3/03
	EPPO guideline No. 170 (1992)	Application of 100 kg product/ha at approx. 30% flowering of *Phacelia*, 28 h before the introduction of bees in the tents (7-day exposure) did not cause adverse effects to honeybees	[redacted],; 2000; M-238790-01-1 KCP 10.3.1.5/01
	Semi-field honey bee study (OEPP/ EPPO Guideline No. 170(4); forced exposure conditions) in flowering apple orchard	No adverse effects on mortality, foraging activity, behaviour, colony strength, amount of brood and food storage after one application of 1200 g a.s./ha/m canopy height (corresponding to 3600 g a.s./ha/3 m canopy height).	[redacted],; 2015; M-528978-01-1 KCP 10.3.1.5/02
	Semi-field honey bee study (OEPP/ EPPO Guideline No. 170(4); forced exposure conditions) in flowering apple orchard	No adverse effects on mortality, foraging activity, behaviour, colony strength, amount of brood and food storage after two applications of 1200 g a.s./ha/m canopy height (corresponding to 3600 g a.s./ha/3 m canopy height) in a 6-day spray interval.	[redacted],; [redacted]; [redacted]; 2015; M-533329-01-1 KCP 10.3.1.5/03

Test substance	Test species/ study type	Endpoint	References
Fosetyl-Al	Bumble bee, 48 h	LD_{50}– contact > 250 µg a.s./bumble bee	[redacted], S.; 2015; M-525339-01-1; KCA 8.3.1.1.2/06

- Studies written in grey typeface are referring either to studies in the corresponding Baseline Dossier for the active substance or the old representative formulation for Annex I inclusion of fosetyl under Directive 91/414/EEC (which is provided for approval renewal as well); whereas studies in black typeface are studies of the Supplementary Dossier for the active substance or the representative formulation Fosetyl-Al WG 80.

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%.

Bold: endpoint used for risk assessment

Risk assessment for bees

The risk assessment for bees is based on the application rate of fosetyl-aluminium (fosetyl-Al) with 3600 g a.s./ha for applications in orchards using the endpoints (LD_{50} values) for fosetyl-Al and its metabolite phosphonic acid.

Hazard Quotients

The risk assessment is based on Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha or in g total substance/ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee or in µg total substance/bee).

Q_H values are calculated using data from the studies performed with the active substance and with the formulation. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honey bees.

Hazard Quotient, oral:

$$Q_{HO} = \frac{\text{maximum application rate}}{LD_{50}\ \text{oral}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\text{µg a.s./bee or µg total substance/bee}]}$$

Hazard Quotient, contact:

$$Q_{HC} = \frac{\text{maximum application rate}}{LD_{50}\ \text{contact}} = \frac{[\text{g a.s./ha or g total substance/ha}]}{[\text{µg a.s./bee or µg total substance/bee}]}$$

Table 10.3.1-2: Hazard quotients for bees – oral exposure

Compound	Oral LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Fosetyl-Al	>110.4	3600	<32.6	50	yes
Phosphonic acid	>848.0	2301 *	<2.9	50	yes

* assuming a quantitative conversion of the parent to the metabolite, 3.6 kg fosetyl-Al corresponds to 2.501 kg H_3PO_3 based on a molar mass of 354.1 g/mol for fosetyl-Al and 82.0 g/mol for H_3PO_3 and assuming that 1 mol fosetyl-Al degrades to 3 mol H_3PO_3

The hazard quotients for oral exposure are below the validated trigger value for higher tier testing (i.e. Q_{HO} < 50).

Table 10.3.1- 3: Hazard quotients for bees – contact exposure

Compound	Contact LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Fosetyl-Al	>100.0	3600	<36.0	50	yes
Phosphonic acid	>1050.0	2501 *	<2.4	50	yes

* assuming a quantitative conversion of the parent to the metabolite, 3.6 kg fosetyl-Al corresponds to 2.501 kg H_3PO_3, based on a molar mass of 354.1 g/mol for fosetyl-Al and 82.0 g/mol for H_3PO_3, and assuming that 1 mol fosetyl-Al degrades to 3 mol H_3PO_3

The hazard quotients for contact exposure are below the validated trigger value for higher tier testing (i.e. $Q_{HC} < 50$).

Further considerations for the risk assessment

In addition to acute laboratory studies with adult honey bees, fosetyl-aluminium (fosetyl-Al) was further subjected to topical acute bumble bee testing (KCA 8.3.1.1.2/06; [redacted] S.; 2015; M-525339-01-1). The study resulted in an LD_{50} of > 250 µg a.s./bumble bee and did not reveal sensitivity differences between honey bee and bumble bee foragers.

Moreover, fosetyl-Al was further subjected to chronic laboratory testing with adult honey bees (KCA 8.3.1.2/01; [redacted], A.; 2015; M-527665-01-1).
This chronic study was designed as a dose-response test by exposing adult honey bees for 10 consecutive days to nominal concentrations of 46.88, 93.75, 187.5, 375 and 750 mg fosetyl-Al/kg feeding solution. The actual test was conducted by using the formulated product Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80). After exposing honey bees for ten consecutive days exclusively to sugar solution containing fosetyl-Al at the respective treatment levels, the 10 day LC_{50} (Lethal Concentration) was determined to be > 750 mg fosetyl-Al/kg, which corresponds to a LDD_{50} (Lethal Dietary Dose) of > 37.3 µg a.s./bee/day. The respective NOEC (No Observed Effect Concentration) for mortality was determined to be 750 mg fosetyl-Al/kg, which corresponds to the NOEDD (No Observed Effect Dietary Dose) of > 37.3 µg a.s./bee/day.

In order to reveal whether fosetyl-Al poses a risk to immature honey bee life stages, a bee brood feeding study (KCA 8.3.1.3/01; [redacted], C.; 2015; M-508986-01-2) has been conducted by following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992), which require, amongst other parameters to "*…use formulated products only… products are fed at a concentration recommended for high-volume use…"*. The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology.
This particular study was conducted with Fosetyl-Al WG 80 and the actual test concentration of fosetyl-Al was 2.4 g a.s./L (2.97 g Fosetyl-Al WG 80/L). 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, the Brood Termination Rates of the test item treatment were overall on a low to moderate level with 27.3, 11.3 and 11.0% for eggs, young larvae and old larvae, respectively. Yet, as compared to the Brood Termination Rates in the control (13.3, 3.7 and 1.7% for eggs, young larvae and old larvae respectively), a slight but statistically significant increase was detected for the test item at the end of the brood observation period. However, neither Brood Indices nor Brood Compensation Indices were significantly increased in the test item as

compared to the control for any brood stage, indicating that these indices performed comparable to the control, including compensations of previous brood losses.

All in all, it can be concluded from the acute and chronic laboratory studies in adult honey bees as well as from the bee brood feeding study (Oomen *et al.*, 1992) investigating side-effects on immature honey bee life stages, that fosetyl-Al is of moderate, general intrinsic toxicity to honey bees.

In order to clarify whether the moderate, general intrinsic bee toxicity of fosetyl-Al poses a risk to honey bee brood and colony development in particular as well as on honey bees in general under realistic worst-case conditions, a higher tier semi-field honey bee brood study (according to the provisions of the OECD Guidance Document 75) was conducted in 2014 under forced/confined exposure conditions, by application of 3600 g a.s./ha as well as a rate of 570 g a.s./ha (spray drift rate) under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia* (KCA 8.3.1.3/02; [redacted], B.; 2015; M-526896-01-1).
The study included four treatment groups: Control (tap water), Test item 1 (3600 g a.s./ha), Test item 2 (570 g a.s./ha) and Reference item (300 g fenoxycarb/ha) with all applications being carried out with a spray volume of 400 L water/ha. For all treatment groups, four replicates (tunnels) were set up. The application of all treatments was conducted during daily bee flight activity at the time of full flowering of the crop. Thereafter, the bees were kept for 7 days within the tunnels (confined exposure phase) and in the evening of the 7th day after application (after bee flight activity) the colonies were relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural area for further monitoring (day 8 to day 27 after treatment). Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Daily mortality assessments were continued along with behaviour around the hive during the post-exposure observation period (day 8 to day 27 after treatment). Colony assessments (food stores, brood areas, colony strength) were made before confinement, after confinement and at the end of the study. Detailed brood assessments (brood termination rate, brood index and brood compensation index) by employing digital photo imaging technology, investigating the fate of more than 200 individually marked cells was performed on 5 occasions throughout the study, covering an entire brood cycle of honey bees.
The application of fosetyl-Al at the rate of 3600 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia* did not cause any adverse effects on mortality, flight intensity, brood development (brood termination rate: 36.5%, brood index: 3.2, compensation index: 3.8 in test item compared to the control with brood termination rate: 41.2%, brood index: 2.9, compensation index: 3.5), as well as on colony strength and brood and food abundance. The application of fosetyl-Al at the rate of 570 g a.s./ha did not cause adverse effect on mortality, flight intensity, colony strength and brood and food abundance but resulted in unclear findings on brood development (brood termination rate: 79.9%, brood index: 1.0, compensation index: 2.1). Since effects were only seen at the lower test rate of 570 g a.s./ha tested in this study but not at all in the higher application rate of 3600 g a.s./ha, the investigation of the lower test rate was repeated in a second study conducted in 2015.

The study conducted in 2015 (KCA 8.3.1.3/03; [redacted], B.; 2015; M-528899-01-1) was performed following the same study design as in 2014. In the repeat study for the lower rate of 570 g a.s./ha no adverse effects on mortality, flight intensity, behaviour, brood development (brood termination rate: 36.1%, brood index: 3.2, compensation index: 3.7 in test item compared to the control with brood termination rate: 29.6%, brood index: 3.5, compensation index: 4.1) as well as on colony strength and brood and food abundance were determined. Thus, this study confirms that fosetyl-Al has no overall adverse effect on brood development at the rate of 570 g a.s./ha.

To complete the data set, two further semi-field studies following the EPPO Guideline No. 170 (4) design were performed, in which Fosetyl-Al WG 80 was applied in flowering apple orchards at the rate of 3600 g a.s./ha (for trees with 3 m canopy height).

The first study (KCP 10.3.1.5/02; [redacted], B.; 2015; M-528978-01-1) included three treatment groups: Control (tap water), Test item (3600 g fosetyl-Al/ha/3 m canopy height, corresponding to 1200 g a.s./ha/m canopy height) and Reference item (400 g dimethoate/ha/3 m canopy height, corresponding to 133.3 g a.s./ha/m canopy height) with applications being carried out with a spray volume of 1350 L water/3 m canopy height, corresponding to 450 L water/ha/m canopy height. For all treatment groups, four replicates (tunnels) were set up. The application was conducted during daily bee flight activity onto the flowering crop at BBCH 63. Thereafter, the bees were kept for 7 days within the tunnels (confined exposure phase) and in the evening of the 7th day after application (after bee flight activity) the colonies were relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural area for further monitoring (day 8 to day 21 after treatment). Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Daily mortality assessments were continued along with behaviour around the hive during the post-exposure observation period (day 8 to day 21 after treatment). Colony assessments (food stores, brood areas, colony strength) were performed once before and once during confinement, and six times after confinement up to the end of the study 45 days after application.
Despite the observation of slightly reduced foraging activity 3 and 6 days after the application (due to a phytotoxic effect by the test item on the blossoms which resulted in reduced attractiveness of blossoms and therefore led to lower foraging intensity), the application of Fosetyl-Al WG 80 at the rate of 3600 g a.s./ha (1200 g a.s./ha/m canopy height) under tunnel conditions in a full flowering apple orchard did not result in unacceptable effects on honey bee mortality, foraging activity, behaviour, colony strength, amount of brood and food storage.

The second study (KCP 10.3.1.5/03; [redacted], J.; [redacted], N.; [redacted], V.; 2015; M-533329-01-1) included three treatment groups: Control (tap water), Test item (3600 g fosetyl-Al/ha/3 m canopy height, corresponding to 1200 g a.s./ha/m canopy height) and Reference item (400 g dimethoate/ha/3 m canopy height, corresponding to 133.3 g a.s./ha/m canopy height) with all applications being carried out with a spray volume of 1500 L water/3 m canopy height, corresponding to 500 L water/ha/m canopy height. For all treatment groups, five replicates (tunnels) were set up. The two applications were conducted in a 6-day interval during daily bee flight activity onto the flowering crop at BBCH 63-65 and BBCH 66-67. The bees were kept for 8 days within the tunnels (confined exposure phase) and in the evening of the 8th day after the 1st application (after bee flight activity) the colonies were relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural area for further monitoring (day 9 to day 22 after treatment). Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Daily mortality assessments were continued along with behaviour around the hive during the post-exposure observation period (day 9 to day 22 after treatment). Colony assessments (food stores, brood areas, colony strength) were performed once before the 1st application and twice after the 1st application during confinement, and five times after confinement up to the end of the study 42 days after treatment.
Despite the observation of slightly reduced foraging activity on days 1 and 6 after the 1st application, the two applications of Fosetyl-Al WG 80 at the rate of 3600 g a.s./ha (1200 g a.s./ha/m canopy height) in a 6-day interval under tunnel conditions in a full flowering apple orchard did not result in unacceptable effects on honey bee mortality, foraging activity, behaviour, colony strength, amount of brood and food storage.

Both studies simulating (repeated) applications at full flowering apple orchards in a semi-field design confirmed that the application of Fosetyl-Al WG 80 at rates of 3600 g fosetyl-Al/ha in full flowering orchards does not result in any unacceptable effects on mortality, foraging activity, behaviour, colony strength, amount of brood and food storage.

Synopsis

Fosetyl-Al and Fosetyl-Al WG 80 are of low acute toxicity to honey bees, with LD_{50} (oral and contact) above the highest tested dose levels (oral: LD_{50}> 110.4 µg a.s./bee, contact: LD_{50}> 100 µg a.s./bee).

The calculated Hazard Quotients for fosetyl-Al are below the validated trigger value which would indicate the need for a refined risk assessment; no adverse effects on honey bee mortality are to be expected at the maximum envisaged fosetyl-Al application rate. This conclusion is confirmed by the results of the bee brood feeding study as well as by the results of the semi-field studies, which covered the maximum application rate of 3600 g a.s./ha.

The acute laboratory study conducted with bumble bees revealed no sensitivity differences between honey bee and bumble bee foragers.

It can be concluded from the acute and chronic laboratory studies in adult honey bees as well as from the bee brood feeding study (Oomen *et al.*, 1992), investigating side-effects on immature honey bee life stages that fosetyl-Al is of a moderate general intrinsic toxicity to honey bees.

Regarding potential side effects of fosetyl-Al on immature honey bee life stages, the conducted bee brood feeding study (Oomen *et al.*, 1992) found slightly to moderately, but statistically significantly increased termination rates of eggs, young and old larvae. Despite of this observation, the brood index and brood compensation indices displayed a continuous increase without any statistical significant difference to the control, indicating a successful development of the brood. Overall the study revealed no ecologically adverse effects on the survival of adult bees and pupae, behaviour, colony strength and overall colony conditions. Thus, when considering the severity of the exposure situation in this worst-case screening test in combination with the absence of effects on both, colony level parameters and also on the overall development of bee brood, it can be concluded even on the basis of this worst-case screening study that the use of fosetyl-Al does not pose an unacceptable risk for adult honey bees, immature honey bee life stages and honey bee colonies.

In order to clarify whether the conclusions on the basis of lower tiered honey bee studies are correct, fosetyl-Al was subjected to confined semi-field testing (according to the provisions of OECD Guidance Document No. 75), by applying the two rates of 3600 and 570 g a.s./ha for Fosetyl-Al WG 80 to full-flowering *Phacelia* during honey bees actively foraging on the crop. This study design is from an apidological and apicultural point of view more realistic than an in-hive feeding of the test compound via a treated sugar solution, which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration). The results of this first higher tier semi-field study confirmed the conclusions made above on the basis of the outcome of the lower-tiered studies, as no adverse direct or delayed effects on mortality of worker bees or pupae, foraging activity, behaviour, nectar- and pollen storage, colony strength, colony development as well as the development of bee brood were observed for the higher test rate of 3600 g a.s./ha, even under aggravated, forced exposure conditions and by digitally following-up in a very detailed manner the fate of individually marked brood cells (digital photographic assessment) from egg stage until emergence. In the same study the application of fosetyl-Al at the rate of 570 g a.s./ha did not cause adverse effect on mortality, flight intensity, colony strength and brood and food abundance. However, unclear findings were determined on brood development. In a repeated test following the same study design the absence of adverse effects on these assessment parameters together with the absence of adverse effects on the development of brood for the rate of 570 g a.s./ha was confirmed. Thus, this study confirms that fosetyl-Al has no overall adverse effect on brood development at the rate of 570 g a.s./ha.

In addition two semi-field studies following the EPPO Guideline No. 170 (4) design were performed, in which Fosetyl-Al WG 80 was applied in flowering apple orchards at the rate of 3600 g a.s./ha (for trees with 3 m canopy height). Both studies simulating exposure of honey bees after (repeated) applications in full flowering apple orchards in a semi-field design confirmed that the application of Fosetyl-Al WG 80 at rates of 3600 g fosetyl-Al/ha does not result in any unacceptable effects on mortality, foraging activity, behaviour, colony strength, amount of brood and food storage.

Conclusions
Overall, it can be concluded that Fosetyl-Al WG 80, when applied at the maximum application rate of 3600 g a.s./ha even during the flowering period of a bee-attractive crop, does not pose an unacceptable risk to honey bees and honey bee colonies.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to 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. As an overview the original summary from the DAR is given below (KCP 10.3.1.1.1/01 and KCP 10.3.1.1.2/01; [redacted], S.; 1999; M-184602-01-1).

One additional study on acute toxicity to bees 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 fosetyl approval renewal. This study is summarized below ([redacted]; [redacted]; 2014; M-503644-01-1).

Report:	[redacted]; [redacted]; 1999; M-184602-01-1
Title:	Laboratory testing for toxicity (acute contact and oral LD50) of EXP10369F on honey bees (*Apis mellifera* L.) (Hymenoptera, Apidae)
Report No.:	R011801
Document No.:	M-184602-01-1
Guideline(s):	EPPO: 170, (1992); Equivalent to US EPA OPPTS Guideline No. 850.3020
Guideline deviation(s):	see page 18
GLP/GEP:	**yes**

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

Oral LD_{50} - 48 h >440 µg product/bee
Contact LD_{50} - 48 h > 390 µg product/bee

Methods:
The test substance was EXP10369F (794 g fosetyl-Al/kg). The study design included 7 experimental groups. Each group had 3 replicates with 10 bees per replicate. EXP10369F was applied at the following nominal doses (both tests): 390, 278, 199, 142 and 102 µg product/bee (equivalent to 310, 221, 158, 113 and 81 µg fosetyl-Al/bee). The average doses measured (oral test) were 440, 333, 246, 164 and 122 µg product/bee (equivalent to 349, 264, 195, 130 and 97 µg fosetyl-Al/bee). In addition to the EXP10369F-treated groups, one negative control and one positive control with a toxic standard (dimethoate) groups were used.

Results:

Oral LD_{50} - 48 h > 440 µg EXP10369F/bee
Contact LD_{50} - 48 h > 390 µg EXP10369F/bee

- **Comments (RMS):** acceptable

Report:	[redacted]3; [redacted]; 2014; M-503644-01-1
Title:	Effects of fosetyl-aluminium WG 80 W (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory
Report No.:	91831035
Document No.:	M-503644-01-1
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 Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) 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 WG 80: Fosetyl-aluminium (LS 74783), 81.0% w/w (analysed); Specification No.: 102000024225 – 01, Batch ID: EV36003202, TQX10146-00.
Under laboratory conditions *Apis mellifera* 50 worker bees per dose were exposed for 48 hours to a single nominal dose level of 100.0 µg a.s. per bee (123.5 µg Fosetyl-Al WG 80/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 measured dose of 110.4 µg a.s. per bee (136.3 µg Fosetyl-Al WG80/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% w/v sucrose solution (oral test).

Dates of experimental work: April 01, 2014 – April 04, 2014

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 – 0.30 µg a.s./bee	0.29 µg a.s./bee
LD_{50} of Reference Item (24 hours) - Oral Test	0.10 – 0.35 µg a.s./bee	0.17 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.
The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.29 and 0.17 µg a.s./bee, respectively.

Toxicity to Honey Bees; laboratory tests

Test Item	Fosetyl-Al WG 80	
Test Species	*Apis mellifera*	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sucrose solution)
Application rate µg a.s./bee	100.0	110.4
LD_{50} µg a.s./bee	> 100.0	> 110.4
LD_{20} µg a.s./bee	> 100.0	> 110.4
LD_{10} µg a.s./bee	> 100.0	110.4
NOED µg a.s../bee*	≥ 100.0	≥ 110.4

* The NOED was estimated using Fisher's 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	2.0	15.0	56.0	4.0	62.0	4.0
0.20	0.0	2.0	20.0	4.0	34.0	0.0
0.15	0.0	0.0	18.0	0.0	28.0	2.0
0.10	0.0	0.0	4.0	0.0	4.0	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						
110.4	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						
0.32	18.0	58.0	92.0	4.0	96.0	2.0
0.16	2.0	6.0	50.0	6.0	60.0	0.0
0.08	0.0	0.0	8.0	0.0	8.0	2.0
0.05	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 = 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 and in the control group (water + 0.5 % Adhäsit), respectively. No test item induced behavioural effects were observed at any time in the contact toxicity test.

Oral Test:
In the oral toxicity test, the maximum nominal test level of Fosetyl-Al WG 80 (*i.e.* 100 µg a.s./bee) corresponded to an actual intake of 110.4 µg a.s./bee. At this dose level and in the control group (50% w/v sucrose solution = 500 g sucrose/L tap water) no mortality occurred after 48 hours, respectively. In the oral test, no behavioural abnormalities occurred at any time in the oral toxicity test.

Conclusions:
The toxicity of Fosetyl-Al WG 80 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 > 110.4 µg a.s./bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to Section CP 10.3.1.1.1.
Additionally, an acute contact toxicity study was conducted on bumble bees with fosetyl-aluminium; the corresponding summary is provided in Document MCA, Section 8.3.1.1.2 (KCA 8.3.1.1.2/06, [redacted], S.; 2015; M-525339-01-1).

CP 10.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with Fosetyl-aluminium WG 80; the corresponding summary is provided in Document MCA, Section 8.3.1.2 (KCA 8.3.1.2/01, [redacted], A.; 2015; M-527665-01-1).

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A honey bee brood feeding study according to the method of Oomen *et al.* 1998 (KCA 8.3.1.3/01, [redacted], C.; 2015; M-508986-01-2) has been conducted with Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) and is included in Document MCA, Section 8.3.1.3.
Two semi-field honey bee brood studies (according to OECD 75) (KCA 8.3.1.3/02, [redacted], B.; 2015; M-526896-01-1, and KCA 8.3.1.3/03, [redacted], B.; 2015; M-528899-01-1) have been conducted with the Fosetyl-Al WG 80 and are included in Document MCA, Section 8.3.1.3.

CP 10.3.1.4 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.

CP 10.3.1.5 Cage and tunnel tests

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

Report:	KCP 10.3.1.5/01 [redacted]; 2000; M-238790-01-1
Title:	Assessment of the side effects of soil applications of EXP 10369F (fosetyl-Al 80 percent w/w) on the honey bee (*Apis mellifera* L.) under semi-field conditions
Report No.:	B003137
Document No.:	M-238790-01-1
Guideline(s):	EPPO guideline No. 170 (1997); Equivalent to US EPA OPPTS Guideline No. 850.3040
Guideline deviation(s):	see page 21-22
GLP/GEP:	**yes**

Endpoint according to EFSA Scientific Report (2005) 54, 1-78 for fosetyl-Al:
A semi field test at 80 kg EXP10369F/ha. No effect on mortality, bee flight intensity and foraging activity, bee brood development and general behavior.

Methods:
The effect of the test substance EXP10369F (79.5% fosetyl-Al, w/w) was examined on small bee colonies in cages placed over field plots with flowering *Phacelia tanacetifolia*.
Three experimental groups, with three replicates each were tested. A cage with one colony was considered as a replicate. In one group the test substance EXP10369F was applied to soil twice at a rate equivalent to 40 kg a.s./ha. The 1st application was performed 1 day before sowing of *Phacelia*. The 2nd application was performed at approximately 30% flowering of *Phacelia* (8 weeks after the 1st application, 28 h before the introduction of bees in the tents). In the 2nd group, the test substance was applied twice at a rate equivalent to 80 kg a.s./ha with the same application timing as in the 1st group. A 3rd group was treated with water at the same timing as in the test substance treated groups. The spray volume was 2 l/m^2.
Mortality, flight and foraging activity, behaviour, and condition of the colonies and the development of the bee brood were assessed. The influence of the test substance was evaluated by comparing the bees in the pesticide-treated cages to those in the control cages treated with water regarding the following observations: (a) mortality at the edge of the treated area and in the bee traps, (b) light intensity (number of flying bees/m^2 flowering *Phacelia* crop), (c) foraging activity, *i.e.*, number of forager bees/m^2 flowering *Phacelia* crop, (d) behaviour of the bees on the crop and around the hive, and (e) development of the bee brood.

Results:

(a) Effect on honey bee mortality
No increased number of dead bees in the dead bee trap could be noticed in the treatment groups T1 and T2 in comparison to the control. The number of dead bees on the linen/tent on d 1 after introducing bees in tents was increased in groups T1 (49.3) and T2 (57.7) in comparison to the number of dead bees on the linen/tent in the control group (20.7). On the following days, no differences in the number of dead bees between the test substance treatments and the water treated control which could be attributed to the influence of the test substance. Over the complete period of exposure (7 d), the average number of dead bees/tent/day did not significantly differ in the treatment group T1 and T2 compared to the control.

(b) Effects on honey bee flight intensity and foraging activity were assessed as follows:

	T 1	T2	Control
Flight intensity (flying; bees per m^2/day)	2.2 ± 1.7 (-)	1.9 ± 1.3 (-)	2.1 ± 1.5 (-)
Foraging activity (forager bees per m^2/day)	10.6 ± 6.0 (+) *	8.0 ± 5.2 (-)	8.1 ± 6.1 (-)
(-): Non-significant compared to the control; (+): significant compared to the control * The increased average number of forager bees in T1 are regarded to be due to a higher density of flowering *Phacelia.*			

(c) Effects on honey bee brood development:
In the bee brood development no abnormal difference which could be attributed to the influence of the test substance were observed between the test substance and control treatments.

(d) Behaviour of the bees:
No abnormal difference in behaviour of the bees was observed between the test substance treatments and the control treatments at any time during the period of assessment.

(e) Conclusion:
Repeated applications of EXP10369F as a localised soil application up to rates equivalent to 0 kg a.s./ha did not cause adverse effects to honeybees in this semi-field study.

- **Comments (RMS):** acceptable. However, the study is more appropriate to evaluate the potential impact of the metabolite HPO_3 rather than that of the active substance.

Report: KCP 10.3.1.5/02[redacted]; 2015; M-528978-01-1
Title: Assessment of side effects of fosetyl-AL WG 80 on the honeybee (*Apis mellifera* L.) in the semi-field after application on flowering apple trees in Germany 2015
Report No.: S15-01634
Document No.: M-528978-01-1
Guideline(s): OEPP/EPPO Guideline No. 170(4), 2010 (modified)
Guideline deviation(s): no major deviations
GLP/GEP: **yes**

Objective:
This study was designed to determine the potential side-effects of Fosetyl-Aluminium WG 80 (Fosetyl-Al WG 80) on the honeybee (*Apis mellifera* L.) after one application on apple trees at full-flowering in Germany in a semi-field study. The evaluation of the treatment effects focused on mortality, flight intensity, behaviour and condition of the colonies.

Materials and Methods:
Test item: Fosetyl-Al WG 80; Sample description: Specification No.: 102000024225, TOX10884-00; Batch-ID: EV36003889; content of fosetyl-aluminium (nominal): 80% w/w, (analysed): 80.5% w/w.

The study included three treatment groups with four replicates (tunnels) each: one tap-water treated control group, one test-item group and one reference item group.
Applications to the trees were made at full-flowering (BBCH 63) with honeybees actively foraging on the crop. The target application rate of the test item Fosetyl-Al WG 80 was 1200 g a.s./ha/m canopy height (CH) (actual rate applied 1214 g a.s./ha/m CH). Tap water was applied in the control group and Perfekthion was applied at a target rate of 133.3 g a.s./ha/m CH in the reference item group (corresponding to 333.3 mL product/ha/m CH). The spray volume was 450 L/ha/m CH.
The initial mean colony sizes per treatment group were in the range of 4550 to 4908 bees. The honeybees remained in the tunnels for 10 days and colonies were assessed once before set-up, once during and six times after the end of the confined phase.

The following endpoints were assessed:

- Mean number of dead bees (worker and pupae separately) on the linen sheets in tunnels and in the dead bee traps before as well as after the application.
- Flight intensity (mean number of forager bees/apple tree row/min before as well as after the application.
- 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).

Dates of experimental work: 20 April 2015 – 08 June 2015

Results:

Mortality

Mortality: Findings are summarized in the table below.

Treatment group		Control (C)	Test item (T)	Reference item (R)
Daily mean mortality (dead worker bees/colony) ± STD	2DBA to 0DBA	58.8±44.1	91.7±39.4	58.2±53.9
	0DAA	13.3±9.5	20.3±8.1	116.8*±70.8
	0DAA to 7DAA	9.4±5.5	13.6±6.7	40.3*±14.5
	0DAA to 21DAA	9.6±3.5	8.4±2.8	29.2*±16.7
Daily mean mortality (dead pupae/colony) ± STD	2DBA to 0DBA	0.1±0.2	0.6±0.8	0.0±0.0
	0DAA	0.0±0.0	2.3*±1.7	1.0±1.2
	0DAA to 7DAA	0.2±0.2	0.7±0.6	0.5±0.3
	0DAA to 21DAA	0.3±0.2	1.0±0.9	0.4±0.1

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

* statistically significantly higher than control group

Throughout the pre-exposure period, mortality of honeybees was slightly but not statistically significant higher in the test item treatment group (T), compared to the control group (C) and the reference item group (R).

During exposure period from day 0 until day 4 after application, mortality across the treatment groups C and T was similar indicating no effect of the test item. Towards the end of the confinement of the bees in the tunnels on days 5 and 6 after application, the mortality in T was statistically significant higher compared to the control group (C) (t-Test pooled, one sided, α=0.05). The higher mean value on 5DAA is mainly influenced by one out of the four replicates (Tb). Therefore, it is unlikely that the difference detected by the statistical analysis is related to an effect of the test item. Furthermore, the mean number of dead bees assessed in T falls in the same range at both days as the mortality observed on other assessment days in T as well as C, indicating that the observed mortality is not biologically meaningful.

During the entire period after the application (0DAA to 21DAA), the mean daily mortality was on the same level in C and T. Therefore, overall, no biologically relevant adverse effect on mortality was found in T.

During the entire period after the application (0DAA to 21DAA), a low number of dead pupae was recorded during the mortality assessments across all treatments (average sum of dead pupae per colony: 0.3, 1.0 and 0.4 for C, T, and R, respectively). The mean number of dead pupae recorded throughout the post-exposure period was not statistically significant different between the treatment groups. At the monitoring site (8DAA to 21DAA), some daily fluctuations occurred but except on 12DAA (R) and on 14DAA (T), no statistically significant difference was observed (t-Test, method Satterthwaite, one-sided, α = 0.05).

<u>Flight Intensity</u>
Findings are summarized in the table below.

Treatment group		**Control (C)**	**Test item (T)**	**Reference Item (R)**
Daily mean flight intensity (bees/min) ± STD	2DBA to 0DBA	14.3±2.9	14.5±3.5	12.6±1.2
	0DAA	26.6±10.4	20.0±3.6	3.0*±1.9
	0DAA to 7DAA	16.7±5.0	11.9±2.0	0.8*±0.2

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 (2DBA and 0DBA).
On the day of application (0DAA) the mean number of foraging bees in T and R was lower compared to C. However, only the mean value in R was statistically significant different from the mean number of foraging bees in C (t-Test, method pooled, one-sided, α = 0.05).
From 1DAA to 6DAA, foraging activity was clearly reduced in the reference item group (R) compared to the control group (C) (t-Test, method pooled and method Satterthwaite, one-sided, α = 0.05; Mann Whitney exact test, one-sided, α = 0.05). During this period, the mean number of foraging bees in T was slightly lower than in C. A statistically significant difference was observed on 3DAA and 6DAA (t-Test, method pooled, one-sided, α = 0.05). As the test item caused a phytotoxic effect on the blossoms of the apple trees (first signs of phytotoxicity appeared on 3DAA), it is probable that the overall lower foraging intensity observed in T was due to the reduced availability of food due to the damaged blossoms. Also on 4DAA and 5DAA, the flight intensity was lower in T compared to C although no statistically significant difference was detected.
Overall, a slight test-item related effect on flight intensity was observed, which was due to the reduced attractiveness of blossoms caused by the phytotoxicity of the test item.

<u>Behaviour of the Bees</u>
From 0DAA to 7DAA, the behaviour of the bees was similar in the treatment groups C and T. During the exposure phase (0DAA to 7DAA) the number of bees showing unusual behaviour was low in both treatments. In total, five bees with locomotion problem were observed in T during this period. As only a small number of honeybees showed the symptoms two days and six days after the end of application, the observed behaviour is not interpreted as test item related or biologically relevant. In the reference item group, a high number of honeybees showing unusual behaviour was observed from 0DAA to 3DAA.

Strength of the Colonies

The overall development of colony strength (mean number of bees per hive) of all treatment groups showed fluctuations in a typical and normal range. The colony strength in the test item group T was on approximately the same level at the first two colony assessments. From the third assessment (11DAA) to the sixth assessment (32DAA), the number of bees/colony was lower in T and R, compared to C. A significant difference between C and T was observed on 11DAA (t-Test, method pooled, one-sided, $\alpha = 0.05$). However, the high colony strength in the control group (C) is in part due to the colony Ca, which had clearly more bees compared to the other replicates. Since the replicate Ca was fed with sugar solution on 3DAA (during the exposure period), it is likely that the colony had better conditions to compensate the limiting tunnel conditions and therefore more favourable conditions to expand compared to the other control replicates.
Throughout the study, the strength of the colonies in C and T had similar growth trends. Therefore, no test-item related effects on colony strength were observed.

Development of the Brood Area

The mean amount of brood in the colonies (sum of cells containing eggs, larvae, and pupae) was assessed and overall, honeybee brood development in the test item treatment group was not affected when compared to the control.

Development of the Food Storage Area

The mean amount of food stores in the colonies (sum of cells containing nectar and pollen) was assessed and 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 rate corresponding to 1214 g a.s./ha/m canopy height, at full-flowering of apple trees, during daily honeybee foraging activity. The effects on honeybee colonies under confined conditions considering mortality, flight intensity, behaviour, colony strength, amount of food and brood cell development were evaluated.
No biologically relevant test-item related adverse effects on mortality were observed.
A slight reduction in foraging activity was discerned in T on 3DAA and 6DAA due to a phytotoxic effect by the test item on the blossoms which resulted in reduced attractiveness of blossoms and therefore led to lower foraging intensity.
No test-item related adverse effects on behaviour were observed.
The overall honeybee brood development in the test item treatment group T, measured as mean number of cells covered with the different types of brood cells per colony was not affected when compared to the control. Furthermore, no test-item related adverse effects on colony strength and food storage were observed.
Fosetyl-Al WG 80 applied at 1214 g a.s./ha/m canopy height to flowering apple trees in presence of honeybees resulted in reduced foraging activity but did not cause unacceptable effects on mortality, behaviour, colony strength, amount of brood and food storage.

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; M-528978-01-1) 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 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 was performed according to the guideline EPPO 170 (4) that is referenced in the data requirements as set out in Commission Regulation (EU) No 284/2013.

The standard design as detailed by the guideline includes a 2 to 3 day acclimatisation period normally followed by a 7 day direct exposure period that is seen as an appropriate post-treatment exposure period. The final duration inside the tunnels and the timepoint of removal from the tunnels is clearly driven by the actual flowering period of the crop or the confinement of the bees to a limited foraging area. The guideline states “Honeybees from small colonies are forced to forage on a flowering crop in field cages (to provide realistic worst-case exposure).” …“Shortly before the application, the number of foraging bees per m² …. should be recorded. … A foraging density of at least 5 bees per m² is required on bee attractive crops … in order to verify exposure.” Since this study was performed in an apple orchard and blooming apple trees are commonly known as bee-attractive and pollinated by honeybees, the flight intensity was measured in the number of forager bees/apple tree row/min. Counts that took place before the application confirmed the presence of 15.8 bees/min in the control, 20.0 bees/min in test item and 20.2 bees/min in the toxic reference item. Additional evidence on exposure is available for 3 time points on the application day (after application) as well as from further counts up to 7 days after application. Generally, as soon as a blossom is successfully pollinated, nectar flow and availability of pollen stop and fruit development starts. In this study the test item caused a phytotoxic effect with first signs appearing 3 days after application in form of damaged blossoms. Consequently, due to the decreasing number of blossoms available in an enclosed system like a tunnel, the presence of the colonies inside the tunnels for longer than 7 days after application could not be justified and a second application was not feasible.

The use pattern for Fosetyl-Al WG 80 foresees 1-3 applications to be performed in 7-10 day intervals at BBCH 55-85. In reality exposure of bees to two subsequent applications is unlikely since first of all the lifespan of a blossom is limited; once pollinated the nectar flow and pollen production will stop and bee-attractivity is no longer given. And second usually not more than one application will take place during the limited flowering period (BBCH 60-69) of apple trees. This was also the case in the current study where the application was performed at BBCH 63, due to the fact that a sufficiently high number of blossoms has to be available as food supply for the bees. This approach is fully in line with the guideline in which it is stated that “Normally, a single application during flowering will be sufficient ....”

The guideline also states that “The toxic standard is used to confirm that the bees are exposed to the treatment and to calibrate the magnitude of the possible effects under trial conditions”. As recommended in the guideline the present study included dimethoate as a toxic standard, which showed the expected effects on adult mortality and foraging activity. The application in all treatment groups was performed with a calibrated portable knapsack 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 1.3 m/s, no rain occurred on the application day and the deviation from the target application rate was +1.14% in the test item and +2.34% 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 guideline 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.

Report:	KCP 10.3.1.5/03 [redacted],;[redacted];[redacted]; 2015; M-533329-01-1
Title:	Evaluation of potential side effects of fosetyl-Al WG 80 (FEA WG 80 W) on honeybees (*Apis mellifera*) in a semi-field test in an apple orchard in Germany at the location Hoefchen
Report No.:	E 319 4749-7
Document No.:	M-533329-01-1
Guideline(s):	OEPP/EPPO Guideline No. 170 (4), 2010 (modified)
Guideline deviation(s):	no major deviations
GLP/GEP:	**yes**

Objective:

This study was designed to determine the potential side-effects of Fosetyl-Aluminium WG 80 (Fosetyl-Al WG 80) on the honeybee (*Apis mellifera* L.) after two applications in a 6-day interval on apple trees at full-flowering in Germany in a semi-field study. The evaluation of the treatment effects focused on mortality, flight intensity, behaviour and condition of the colonies.

Materials and Methods:

Test item: Fosetyl-Al WG 80; Sample description: Specification No.: 102000024225, TOX10884-00; Batch-ID: EV36003889; content of fosetyl-aluminium (nominal): 80% w/w, (analysed): 80.5% w/w.

The study included three treatment groups with five replicates (tunnels) each: tap water-treated control, reference item and test item. Two foliar applications were performed in a 6-day interval onto full flowering apple trees.

Two applications to the trees were made in a 6-day interval at full-flowering (BBCH 63-65 and BBCH 66-67) with honeybees actively foraging on the crop. The target application rate of the test item Fosetyl-Al WG 80 was 1200 g a.s./ha/m canopy height (CH). Tap water was applied in the control group and Perfekthion was applied at a target rate of 133.3 g a.s./ha/m CH in the reference item group (corresponding to 333.3 mL product/ha/m CH). The spray volume was 500 L/ha/m CH.

The initial mean colony sizes per treatment group w in the range of 2100 to 3255 cm² comb area covered by bees. Apparently healthy, queen-right honeybee colonies, equalised for adult worker bees, brood and food stores as reasonably possible were used for the purpose of this study.

The following assessments and observations were made during the study:

- Mean number of dead bees (worker and larvae/pupae separately) on the water-permeable sheets in tunnels and in the dead bee traps+water permeable sheets in front of hive before as well as after the application.
- Flight intensity (mean number of forager bees/apple tree row/min before as well as after the application.
- 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).

Dates of experimental work: 25 April 2015 – 10 June 2015

Results:

Mortality

Mortality: Findings are summarized in the table below.

Assessment parameter	Assessment period	Control (1)		Toxic reference (2)		Test item (3)	
		mean	SD	mean	SD	mean	SD
Mean Number of Dead Worker Bees	Mean pre-exposure period (-3DAA to 0DAA)	44.3	28.9	36.3	23.9	42.9	30.6
	Mean exposure period (1DAA to 6DAA) [1]	19.5	2.9	90.3	19.0	12.6	5.0
	Mean exposure period (7DAA to 8DAA) [2]	57.0	26.9	248.7	138.2	49.4	31.4
	Mean post-exposure period (9DAA to 21DAA)	2.1	1.6	5.1	6.9	1.4	1.3
Mean Number of Dead Pupae	Mean pre-exposure period (-3DAA to 0DAA)	0.6	1.0	0.4	0.5	0.2	0.4
	Mean exposure period (1DAA to 6DAA) [1]	0.5	0.5	0.8	0.8	0.3	0.4
	Mean exposure period (7DAA to 8DAA) [2]	0.1	0.1	1.4	1.1	0.9	1.0
	Mean post-exposure period (9DAA to 21DAA)	0.2	0.8	0.3	0.9	0.2	0.5

[1]: exposure period after first application [2]: exposure period after second application
DAA: day(s) after first application pre: before first application SD: standard deviation

In honeybee colonies exposed to the test item as well those of the control, mortality was low throughout the entire test period for worker bees, pupae and larvae. The treatment with the test item did not result in any detectable effects on mortality (worker bees, pupae). Statistical analysis revealed no significant differences between the control and the test item group for any of the assessed mortality parameters.

The colonies treated with the reference item showed statistically significant increases in worker bee mortality from 2 to 8DAA and 10 to 11DAA that confirm that the colonies and the test system were adequate to detect effects on honeybee survival.

Foraging Activity
Findings are summarized in the table below.

Assessment parameter	Assessment period	Control (1)		Toxic reference (2)		Test item (3)	
		mean	SD	mean	SD	mean	SD
Mean Number of Foraging Bees	Mean pre-exposure period (-3DAA to 0DAA)	5.3	6.3	5.7	6.4	5.3	6.1
	Mean on day of first application (0DAA post)	13.4	1.1	0.0	0.0	10.0	2.6
	Mean exposure period (1DAA to 6DAA) [1]	21.3	21.0	10.3	9.7	19.7	21.9
	Mean on day of second application (6DAA post) [2]	71.3	10.7	3.3	1.0	63.2	9.6
	Mean on day after second application (7DAA) [2]	70.1	9.1	0.8	0.6	62.9	13.4

[1]: exposure period after first application
[2]: exposure period after second application
DAA: day(s) after first application pre: before first application
SD: standard deviation

Before the first application, foraging activity was very homogenous and no statistically significant differences were detected. In comparison to the control, the foraging activity of honeybees in the test item was at the same level except for 2 days with a slight but significant reduction of foraging bees (0DAApost and 6DAApre application). No statistically significant difference between control and test item was found at any other day. After the second application the foraging activity in the control and the test item group remained at the same high level.
After the first and second application there was a distinct and statistically significant reduction of foraging activity in the toxic reference item resulting in a total cessation immediately after the application and decreased foraging activity from 3DAA onwards.

Behaviour of the Bees
There was no evidence that the test item treatments resulted in adverse effects on behaviour. Neither signs of intoxication or repellence, nor aggressive behaviour, change in the cleaning behaviour or any other form of behavioural change of the bees were observed after the respective application of the test item and thereafter.

Strength of the Colonies
After setup in the tunnels the strength of the honeybee colonies remained on the same level except for the test item group showing an increased number of worker bees compared to the control group on 8DAA. After the release from the tunnels, the strength of the colonies increased in all treatment groups, but was significantly lower in the toxic reference.

Brood Development
Brood development was very homogenous for the honeybee colonies in the control as well as in the test item treatment. The data reflected very well the typical seasonality of brood development in honeybee colonies. The abundance of brood in control and test item remained at the same level from the first to the fourth assessment (-1DAA to 14DAA) and increased afterwards steadily throughout the monitoring period. No statistically significant difference in the development of brood was found between control and test item at any day. Although the abundance of brood was statistically significant lower in the toxic reference item compared to the control at 4DAA, 8DAA and 21DAA, the overall increasing trend was also recorded in this group.

Food Stores

During the confined exposure period the food stores remained on the same level in the control and the test item, while there was a statistically significant decrease of food cells in the toxic reference item group on 8DAA. Pollen and nectar cells showed an increase on the first assessment after confinement on (14DAA) in all treatment groups, but to a significantly slighter extent in the toxic reference item and the test item group compared to the control. While this trend remained in the toxic reference group throughout the monitoring period (up to 42DAA), the food stores in the test item group showed a steady increase. Compared to the control, the food stores in the test item were slightly higher until the end of the monitoring period.

Conclusion:

Fosetyl-Al WG 80 was applied twice in an interval of 6 days at a rate of 4.5 kg Fosetyl-Al WG 80/ha/3 m CH (3600 g a.s./ha/3 m CH) in flowering apple trees during honeybee foraging activity. In this study honeybee foraging activity was assessed for 8 days after the first application as well as mortality and behaviour for 22 days after the first application. Colony strength, development of brood area and food stores were assessed up to 42 days after the first application.

No test-item treatment related adverse effects on adult and pupae mortality were observed.

Foraging activity of the honeybees in the test item group was slightly but statistically significantly reduced compared to the control directly after the first application and directly before the second application but no difference was detected during any other assessment.

No test-item related adverse effects on behaviour were observed.

Colony strength and size of food stores was comparable between control and test item treatment. Honeybee brood development in the test item group was comparable with the control group. Considering the individual brood stages eggs, larvae and pupae some transient differences occurred in-between treatment groups that can be explained by natural variability in these parameters.

Overall, the application of 4.5 kg Fosetyl-AL WG 80/ha/3 m CH (3600 g a.s./ha/ 3 m CH) in a 6-day interval in flowering apple trees in the presence of honeybees did not result in unacceptable effects on honeybee foraging activity, mortality, behaviour, colony strength, brood development and food stores.

Request from the RMS:

Could you please indicate if some data are available to précise the level of exposure of the exposed colony in the semi-field study in an apple orchard ([redacted], J.; [redacted], N.; [redacted], V.; 2015; M-533329-01-1)?

Response from BCS:

The study was performed according to the guideline EPPO 170 (4) that is referenced in the data requirements as set out in Commission Regulation (EU) No 284/2013.

The standard design as detailed by the guideline includes a 2 to 3 day acclimatisation period normally followed by a 7 day direct exposure period that is seen as an appropriate post-treatment exposure period. The final duration inside the tunnels and the timepoint of removal from the tunnels is clearly driven by the actual flowering period of the crop or the confinement of the bees to a limited foraging area. The guideline states "Honeybees from small colonies are forced to forage on a flowering crop in field cages (to provide realistic worst-case exposure)." … "Shortly before the application, the number of foraging bees per m² … should be recorded. … A foraging density of at least 5 bees per m² is required on bee attractive crops … in order to verify exposure." Since this study was performed in an apple orchard and blooming apple trees are commonly known as bee-attractive and pollinated by honeybees, the flight intensity was measured in the number of forager bees/apple tree row/min. Counts that took place before the first application confirmed the presence of 8.7 bees/min in the control, 10.0 bees/min in test item and 11.3 bees/min in the toxic reference item. Additional evidence on exposure is available for 3 time points on the day of the first application (after application) as well as from further counts performed on the 6 days following days. Counts that took place before the second application confirmed the presence of 15.8 bees/min in the control, 9.9 bees/min in test item and

6.3 bees/min in the toxic reference item. Additional evidence on exposure is available for 3 time points on the day of the second application (after application) plus from further counts on the day thereafter. Generally, as soon as a blossom is successfully pollinated, nectar flow and availability of pollen stop and fruit development starts.

The use pattern for Fosetyl-Al WG 80 foresees 1-3 applications to be performed in 7-10 day intervals at BBCH 55-85. In reality exposure of bees to two subsequent applications is unlikely since first of all the lifespan of a blossom is limited; once pollinated the nectar flow and pollen production will stop and bee-attractivity is no longer given. And second usually not more than one application will take place during the limited flowering period (BBCH 60-69) of apple trees. Therefore the current study must be seen as a worst-case simulation in which the first application was performed at BBCH 63-65 followed by a second application that took place 6 days later at BBCH 65-67, which is an even shorter spray interval as in the intended use pattern.

The guideline also states that "The toxic standard is used to confirm that the bees are exposed to the treatment and to calibrate the magnitude of the possible effects under trial conditions". As recommended in the guideline the present study included dimethoate as a toxic standard, which showed the expected effects on adult mortality and foraging activity. The application in all treatment groups was performed with a calibrated motorised Port sprayer simulating a commercial application. Several criteria were established on the condition for performance of the application to ensure appropriate exposure. Wind conditions were described as being calm, no rain occurred during 6 hours after the applications and the previously prepared target amount for the application rate was completely applied inside each tunnel.

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 applications, combined with the description of exposure in the guideline 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.

CP 10.3.1.6 Field tests with honeybees

Not necessary when considering the outcome of the risk assessment and the results of the lower-tiered studies.

CP 10.3.2 Effects on non-target arthropods other than bees

The risk assessment was performed according to Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi *et al.*, 2000[1]).

Table 10.3.2- 1: Fosetyl-Al WG 80: Ecotoxicological endpoints for arthropods other than bees (current representative formulation)

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	
Aphidius rhopalosiphi [redacted]; 1999; M-184606-01-1	WG 80 Laboratory, glass plates.	LR_{50} > 18.56 kg prod/ha; ER_{50} > 18.56 kg prod/ha	
Rep.Nr: R011803		Corr. Mortality [%]	Effect on Reproduction [%]
KCP 10.3.2.1/01	11.88 kg prod/ha	0.0	10.7
	18.56 kg prod/ha	-2.0[A]	8.5
Aphidius rhopalosiphi [redacted]; 2001; M-201953-01-1	WG 80 Laboratory, glass plates	LR_{50} > 80 kg prod/ha; ER_{50} > 80 kg prod/ha Corr. Mortality [%]	Effect on Reproduction [%]
Rep.Nr: C012023	5 kg prod/ha	0	0.2
KCP 10.3.2.1/02	10 kg prod/ha	0	-6.8[B]
	20 kg prod/ha	0	13.6
	40 kg prod/ha	0	15.2
	80 kg prod/ha	2	20.9
Typhlodromus pyri [redacted]D; 1999; M-184622-01-1	WG 80 Laboratory, glass plates		
Rep.No: R011811		Corr. Mortality [%]	Effect on Reproduction [%]
KCP 10.3.2.1/03	11.88 kg prod/ha	77.1	96.8
	18.56 kg prod/ha	86.7	100.0
Typhlodromus pyri [redacted]; 2000; M-238637-01-1	WG 80 Extended Lab., exposure on		
Rep.No: B002979	detached bean leaves	Corr. Mortality [%]	Effect on Reproduction [%]
KCP 10.3.2.1/01	7.43 kg prod/ha	69.2	77.5
	18.56 kg prod/ha	98.9	n.a.
Typhlodromus pyri [redacted]; 2001; M-202973-01-1	WG 80 Laboratory, glass plates	LR_{50} > 18.87 kg prod/ha; ER_{50} > 2.52 kg prod/ha	
Rep.No: CW01/0		Corr. Mortality [%]	Effect on Reproduction [%]
KCP 10.3.2.1/04	2.52 kg prod/ha	4.4[A]	22.1
	8.81 kg prod/ha	1.1	59.3
	18.87 kg prod/ha	1.1	58.4
Typhlodromus pyri [redacted]; 2007; M-295474-01-1	WG 80 Extended Lab., exposure on	LR_{50} > 80 kg prod/ha; ER_{50} > 80 kg prod/ha	
Rep.No: 37191062	detached bean leaves	Corr. Mortality [%]	Effect on Reproduction [%]
KCP 10.3.2.2/02	2.5 kg prod/ha	-2.0[A]	7.9
	5.0 kg prod/ha	20.4	57.6
	11.25 kg prod/ha	-8.2[A]	11.2
	20.0 kg prod/ha	-2.0[A]	43.8
	40.0 kg prod/ha	-8.2[A]	32.3
	80.0 kg prod/ha	-4.1[A]	-20.2[B]

[1] Candolfi *et al.*: Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods; ESCORT 2 workshop (European Standard Characteristics Of Non-Target Arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Coccinella septempunctata [redacted],; 1999; M-184632-01-1 Rep.No: R011815 KCP 10.3.2.1/07	WG 80 Laboratory, glass plates Control 11.88 kg prod/ha 18.56 kg prod/ha	 Corr. Mortality [%] / Eggs/Female/Day - / 12.2 -2.4 / 9.8 78.6 / 19.2
Coccinella septempunctata [redacted],; 2011; M-412084-01-1 Rep.No: 111048020A KCP 10.3.2.2/03	WG 80 Extended Lab., exposure on detached bean leaves Control 12 kg prod/ha 19 kg prod/ha 33 kg prod/ha 58 kg prod/ha 82 kg prod/ha	LR_{50} > 82 kg prod/ha; no effect on reproduction Corr. Mortality [%] / Eggs/Female/Day / Hatching [%] - / 4.1 / 76.3 6.5 / 4.5 / 76.8 3.2 / 3.6 / 76.2 3.2 / 4.1 / 76.1 -3.2[A] / 4.0 / 76.0 -6.5[A] / 4.1 / 76.8
Aleochara bilineata [redacted]O; 2000; M-238636-01-1 Rep.No: B002978 KCP 10.3.2.1/06	WG 80 Laboratory, spray deposits on quartz sand 11.88 kg prod/ha 18.56 kg prod/ha	ER_{50} > 18.56 kg prod/ha Effect on Reproduction [%] 9.8 14.8
Aleochara bilineata [redacted],; 2011; M-413058-01-1 Rep.No: 111048021A KCP 10.3.2.2/04	WG 80 Extended Lab., spray deposits on soil (LUFA 2.1) 36 kg prod/ha 57 kg prod/ha 67 kg prod/ha	ER_{50} > 67 kg prod/ha Effect on Reproduction [%] 7.4 16.8 21.9
Poecilus cupreus [redacted],; 1999; M-184577-01-1 Rep.No: R011705 KCP 10.3.2.1/05	WG 80 Laboratory, spray deposits on sand and beetles Control 12.09 kg prod/ha 18.89 kg prod/ha	LR_{50} > 18.89 kg prod/ha Corr. Mortality [%] / Food consumption [Fly pupae/beetle over 2 weeks] - / 2.5 -3.3[A] / 3.0 -3.3[A] / 3.0
Poecilus cupreus [redacted],; 1999; M-184635-01-1 Rep.No: R011817 KCP 10.3.2.1/08	WG 80, Non-GLP screening, spray deposits on quartz sand control 124 kg prod/ha	Mortality [%] / Food consumption [no. of fly pupae/beetle] 0 / 3.2 0 / 4.3
Pardosa sp [redacted]P; 1999; M-184609-01-1 Rep.No: R011805 KCP 10.3.2.1/09	WG 80, Non-GLP screening, spray deposits on quartz sand 126 kg prod/ha	 Mortality [%] 65
Predatory mites [redacted],; 2010; M-367548-01-1 Rep.No: S09-01000 KCP 10.3.2.4/01	WG 80 Field, in apple orchards, Southern France Trial **01**: 3 x 3.2 kg prod/ha at 2 d spray interval. Trial **02**: 3 x 4.0 kg prod/ha and 3 x 7.2 kg prod/ha at 18-21 d spray interval.	No unacceptable effects on predatory mite populations

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Predatory mites [redacted],; 2013; M-475378-01-1 Rep.No: S13-01518 KCP 10.3.2.4/02	WG 80 Field, in apple orchards central zone T1: 3 x 4.5 kg prod/ha (interval 3-4d) T2: 3 x 7.5 kg prod/ha (interval 7d) T3 3 x 3.75 kg prod/ha (interval 9d)	No unacceptable effects on predatory mite populations

[A]: A negative value indicates a lower mortality in the treatment than in the control
[B]: A negative value indicates a higher reproduction rate in the treatment than in the control
n.a.: not assessed

The non-target arthropod data as presented in the table above indicate that Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) has a low toxicity to *Aphidius rhopalosiphi* (effects on mortality or reproduction under laboratory conditions <50% up to and including 80 kg prod/ha). Effects on mortality of *Coccinella septempunctata* exceeded under laboratory conditions 50% at an application rate of 18.56 kg prod/ha. But under more realistic extended laboratory conditions no adverse effects on mortality or reproduction were observed for *Coccinella septempunctata* up to and including an application rate of 82 kg prod/ha. Effects on the reproduction of *Aleochara bilineata* were under extended laboratory conditions below 22% at an application rate of 67 kg prod/ha.

The studies for the predatory mite *Typhlodromus pyri* need a more detailed evaluation. In the Addendum to the DAR (2009) it has been stated:

"Both the standard and the extended laboratory study by [redacted] ([redacted], A.; 1999; M-184622-01-1 and [redacted], A.; 2000; M-238637-01-1) on *T pyri* revealed severe effects of EXP10369F at 15 kg a.s./ha to this predatory mite. [redacted] ([redacted], P.; 2001; M-202973-01-1), in contrast, found lower mortality, but significant impact on reproduction was observed. Both studies conducted by [redacted] are characterized by an extreme variability regarding the air humidity and/or temperature. These points are addressed as deviations in both studies. Although these measurements refer to the climatic chamber (in which the whole set-up was placed) and not to the test units, it cannot be excluded that the test organisms encountered these environmental variations. In the study performed by [redacted] ([redacted], P.; 2001; M-202973-01-1) the "island-method", a recent improvement of the test design has been applied. The "islands" are thin glass slides floating on a water surface, preventing mites from escape and leads to a very homogenous humidity. Therefore, the result achieved by [redacted] ([redacted], P.; 2001; M-202973-01-1) were more relevant."

It is therefore proposed to base the tier 1 risk assessment on the mortality data from [redacted] ([redacted], P.; 2001; M-202973-01-1) which indicated an LR_{50} of >18.87 kg prod/ha.

To further clarify the situation concerning the effects on predatory mites an additional extended laboratory study with *Typhlodromus pyri* has been conducted in 2007 ([redacted], M.; 2007; M-295474-01-1) in the same laboratory that has conducted before the studies from [redacted] ([redacted], A.; 1999; M-184622-01-1 and [redacted], A.; 2000; M-238637-01-1). This new extended laboratory study from 2007 which was evaluated in the Addendum to the DAR (2009) indicated an LR_{50} and ER_{50} >80 kg prod/ha. In line with the recommendation of the RMS in the Addendum to the DAR from October 2009 it is proposed to use the endpoint of this extended laboratory study ([redacted], M.; 2007; M-295474-01-1) for the tier 2 risk assessment.

Tier 1 in-field risk assessment for other non-target arthropods

Table 10.3.2- 2: Tier 1 in-field risk assessment for non-target arthropods

Crop	Species	Appl. rate [kg prod./ha]	MAF	LR_{50} [kg prod./ha]	HQ	Trigger
Orchards	*T. pyri*	4.5	2.3	> 18.87	< 0.55	2
	A. rhopalosiphi	4.5	2.3	> 80.00	< 0.13	

Tier 1 off-field risk assessment for other non-target arthropods

Table 10.3.2- 3: Tier 1 off-field risk assessment for non-target arthropods

Crop	Species	Appl. rate [kg prod./ha]	MAF	Drift [%]	VDF	Correction factor	LR_{50} [kg prod./ha]	HQ	Trigger
Orchards	*T. pyri*	4.5	2.3	11.01	10	10	> 18.87	< 0.06	2
	A. rhopalosiphi	4.5	2.3	11.01	10	10	> 80.00	< 0.01	2

For *Aphidius rhopalosiphi* and *Typhlodromus pyri* the calculated HQ values for the in-field and off-field scenario are below the trigger of concern, indicating acceptable risk.
To address the question of the reproduction effects >50% that were observed in the *Typhlodromus pyri* study ([redacted], P.; 2001; M-202973-01-1) an tier 2 risk assessment for *Typhlodromus pyri* and two additional species (*C. septempunctata* and *A. bilineata*) is provided below.

Tier 2 in-field risk assessment for non-target arthropods

Table 10.3.2- 4: Exposure assessment for in-field assessment

Crop / no. of applications	Appl. rate [kg prod./ha]	MAF	in-field PEC_{max}. [kg prod./ha]
Orchards / 3	3.6	2.3	8.28

Table 10.3.2- 5: Tier 2 risk assessment for terrestrial non-target arthropods for the in-field scenario

Crop	Species	In-field PEC_{max} [kg prod./ha]	LR_{50}, ER_{50} [kg prod./ha]	Risk acceptable if	Refined risk assessment required
Orchards	*T. pyri*	8.28	>80.0	Effects are < 50%	No
	C. septempunctata		>82.0	Effects are < 50%	No
	A. bilineata		>67.0	Effects are < 50%	No

The tier 2 in-field risk assessment confirms the results of the tier 1 risk assessment and indicates that no unacceptable adverse effects on non-target arthropods are to be expected from the use of Fosetyl-Al WG 80 according to the proposed use pattern.

This conclusion is also confirmed by the results of two predatory mite field studies ([redacted], J.; 2010; M-367548-01-1, and [redacted], D.; 2013; M-475378-01-1) that indicated no unacceptable adverse effects under field conditions with application rates of 3 x 7.2 kg prod/ha and 3 x 7.5 kg prod/ha, respectively.

Tier 2 off-field exposure assessment for other non-target arthropods

Table 10.3.2- 6: Exposure assessment for off-field assessment (Tier 2)

Crop	Application rate [kg prod./ha]	MAF	Drift [%]	Veg. distr. factor	Correction factor	off-field PEC_{max} [kg prod./ha]	Remark
Orchards	3.6	2.3	11.01	10	5	0.198	in case of 2-D study design

Table 10.3.2- 7: Tier 2 risk assessment for terrestrial non-target arthropods for the off-field scenario

Crop	Species	off-field PEC_{max} [kg prod./ha]	LR_{50}; ER_{50} [kg prod./ha]	Risk acceptable if	Refined risk assessment required
Orchards	*T. pyri*	0.198	>80.0	Effects are < 50%	No
	C. septempunctata		>82.0	Effects are < 50%	No
	A. bilineata		>67.0	Effects are < 50%	No

The tier 2 off-field risk assessment confirms the results of the tier 1 off-field risk assessment and indicates that no unacceptable adverse effects on non-target arthropods are to be expected from the use of Fosetyl-Al WG 80 according to the proposed use pattern.

Conclusions
The tier 1 and the tier 2 risk assessment indicated in line with available field data that no unacceptable adverse effects are to be expected for non-target arthropods in the off-field and the in-field habitat from the use of Fosetyl-Al WG 80 according to the proposed use pattern.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report: KCP 10.3.2.1/01 [redacted], 1999; M-184606-01-1
Title: Effects of EXP10369F on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Aphidiidae) in the laboratory
Report No.: R011801
Document No.: M-184606-01-1
Guideline(s): IOBC/WPRS: (1992)
US EPA OCSPP Guideline number 850.SUPP
Guideline deviation(s): none
GLP/GEP: yes

Materials and methods:
Formulated product Fosetyl-Al WG 80 (EXP 10369F, a.s. content 808 g/kg fosetyl-Al, batch no. OP990544). *Aphidius rhopalosiphi*, four replicates, each containing 10 wasps (5 females and 5 males) per treatment group were exposed to fresh dried residues on glass plates at rates of 12.0 and 18.75 kg test item/ha (nominal, 11.88 and 18.56 kg/ha measured) in 200 L water/ha. A tap water control and a toxic reference control (0.85 mL Reflekthion EC in 200 L water/ha) were included in the study design. Mortality and behaviours abnormalities were recorded 1, 2, 24 and 48 hours after test initiation. Reproduction measured as parasitation rate of aphids was recorded 11 days after the 24 hours parasitation period of aphids by the wasps.

Findings:

	Untreated control	Fosetyl-Al WG 80 (EXP10369F)		Toxic standard Perfekthion EC
Application	**Water**	**18.75 kg pr./ha**	**12.0 kg pr./ha**	**336.6 mg dimethoate/ha**
Mortality (%)	5.0	2.5 ^{ns}	5.0 ^{ns}	100 [1)]
Corrected mortality (%)	-	-2.6	0.0	100
Parasitation efficiency, mean ± SD number of aphid mummies per female (n=sample size)	11.8±11.8 (n= 20)	10.8±7.0 ^{ns} (n=20)	7.0±6.9 ^{ns} (n= 18)	-

ns: Differences with the control not statistically significant (Bonferroni-U-test).
[1)] Differences with the control statistically significant (Bonferroni-U-test, α=0.05).

After 48 hours of exposure to the test substance, the mortality in the test substance groups was equal or lower than that in the controls, indicating lack of lethal effects of Fosetyl-Al WG 80 (EXP10369F) on adult animals at the two rates tested. No behavioural effects were observed on adults in the control or test substance groups.
The mean number of aphid mummies produced per female was 11.8, 10.8 and 7.0 for the controls, 18.56 kg product/ha and 11.88 kg product/ha, respectively. Based on these mean data, the estimated reduction in parasitation efficiency using a standard formula is 8.5% and 40.7% for the 18.56 and 11.88 kg product/ha groups, respectively.

Conclusions:
Under the worst-case conditions of this test, spray treatments of Fosetyl-Al WG 80 (EXP10369F) at rates equivalent to 18.75 kg product/ha (nominal, 15 kg a.s./ha) and 11.88 kg product/ha (nominal, 9.6 kg a.s./ha) did not cause adverse lethal or sub-lethal effects on *A.rhopalosiphi*.

This study was already evaluated for the Annex I listing of fosetyl-Al.

❑ **RMS Conclusion: acceptable**

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: Mead-Briggs, M.A. *ET AL.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) in: Candolfi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 13 – 25.

Test endpoints according to current guideline:
- Mortality of adult wasps during 48 hours exposure.
- Fecundity of surviving female wasps over a 24 hours oviposition period.

Exposure according to current guideline:
Treatments are applied to glass plates. When dry, these are used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) are placed in each arena. There are to be a minimum of four replicate arenas (i.e. 40 wasps in total) in each treatment.

Evaluation according to current guideline:
Assessments of treatment effects are made at 2, 24 and 48 hours. To assess any effects on the relative fecundity of the surviving insects, a minimum of 15 surviving females are taken after 48 hours and individually confined over untreated aphid-infested plants. After 24 hours the wasps are removed and the plants are left for a further 10 to 12 days under controlled environmental conditions before the number of aphid mummies that have developed is assessed.

Validity Criteria:

	Guideline	Test result
Control mortality	Not more than 5 out of 40 wasps (12.5%)	5.0%
Toxic reference mortality (according to study protocol)	>50%	100%
Reproduction rate	≥ 5 mummies/female ≤ 2 females producing 0 mummies	11.8 mummies/female 0 female with 0 mummies

Study Remarks:
No differences were found between the current guideline and the actual study. All procedures and assessments were conducted according to the instructions present in the guideline. A slight difference occurred once during the study, with the temperature rising by 0.5 °C by 2 to 3 hours above the agreed limit. However this small increase is not expected to affect he test results. All study parameters and assessments were obtained as described in the guideline.

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline (Mead-Briggs *et al*., 2000). The validity criteria of the current test guideline were fulfilled.

Report:	KCP 10.3.2.1/02 [redacted].; [redacted]; 2001; M-201953-01-1
Title:	Effects of AE F053616 00 WG80 A101 on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Braconidae) in the laboratory - dose response test -
Report No.:	C012023
Document No.:	M-201953-01-1
Guideline(s):	IOBC/WPRS 1988 and current improvements of the ring-test group
Guideline deviation(s):	none
GLP/GEP:	yes

Material and methods
Test item: Fosetyl-Al WG 80 (AE F053616 00 WG80 A101 or EXP 10369F, a.s. content 795 g/kg fosetyl-Al, batch no. OP980907).
Aphidius rhopalosiphi, four replicates, each containing 10 wasps (5 females and 5 males) per treatment group were exposed to fresh dried residues on glass plates at rates of 5, 10, 20, 40 and 80 kg test item/ha in 200 L water/ha. A deionized water control and a toxic reference (0.3 mL Perfekthion EC, equivalent to 125.2 mg dimethoate, in 200 L water/ha) were included in the study design. Mortality and behavioural abnormalities were recorded 2, 24 and 48 hours after test initiation. Reproduction measured as parasitation rate of aphids was recorded 10 or 11 days, respectively after the 24 hours parasitation period of aphids by the wasps.

Findings

Treatment level	Corrected mortality	significance	Parasitation efficiency	significance
Control	0.0		-	
5 kg product/ha	0.0	n.s.	30.2	n.s.
10 kg product /ha	5.0	n.s.	-6.8	n.s.
20 kg product /ha	0.0	n.s.	13.6	n.s.
40 kg product /ha	0.0	n.s.	15.2	n.s.
80 kg product /ha	2.5	n.s.	10.9	n.s.
Toxic standard	100.0	*		

n.s. not significant

Conclusions

The effect of AE F053616 00 WG80 to the parasitoid wasp, *Aphidius rhopalosiphi* under worst-case conditions (glass plate) was determined as follows:

LR_{50}	> 80 kg product/ha
ER_{50}	> 80 kg product/ha

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: Mead-Briggs, M.A. *ET AL.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DESTEPHANI-PEREZ) (Hymenoptera: Braconidae) in: Candolfi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 13 – 25.

Test endpoints according to current guideline:

- Mortality of adult wasps during 48 hours exposure.
- Fecundity of surviving female wasps over a 24 hours oviposition period.

Exposure according to current guideline:
Treatments are applied to glass plates. When dry, these are used to form the floor and ceiling of shallow arenas. Ten adult wasps (including a minimum of five females) are placed in each arena. There are to be a minimum of four replicate arenas (i.e. 40 wasps in total) in each treatment.

Evaluation according to current guideline:
Assessments of treatment effects are made at 2, 24 and 48 hours. To assess any effects on the relative fecundity of the surviving insects, a minimum of 15 surviving females are taken after 48 hours and individually confined over untreated aphid-infested plants. After 24 hours the wasps are removed and the plants are left for a further 10 to 12 days under controlled environmental conditions before the number of aphid mummies that have developed is assessed.

Validity Criteria:

	Guideline	Test result
Control mortality	Not more than 5 out of 40 wasps (12.5%)	0.0%
Toxic reference mortality (according to study protocol)	>50%	100%
Reproduction rate	≥ 5 mummies/female ≤ 2 females producing 0 mummies	44.1 mummies/female 0 female with 0 mummies

Study Remarks:
No differences were found between the current guideline and the actual study. All procedures and assessments were conducted according to the instructions present in the guideline.

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline (Mead-Briggs *et al*., 2000). The validity criteria of the current test guideline were fulfilled.

Report:	KCP 10.3.2.1/03[redacted]1; 1999; M-184622-01-1
Title:	Effects of EXP10369F on the predatory mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) in the laboratory: final report
Report No.:	R011811
Document No.:	M-184622-01-1
Guideline(s):	IOBC/WPRS: (1988)# US EPA OCSPP guideline: 850.3030
Guideline deviation(s):	none
GLP/GEP:	**yes**

Materials and methods:
Formulated product Fosetyl-Al WG 80 (EXP 10369F; a.s. content 508 g/kg fosetyl-Al; batch no. OP990544). *Typhlodromus pyri*, 5 replicates, each containing 20 protonymphs per treatment group, were exposed to fresh dried residues on glass plates at rates of 12.0 and 18.75 kg test item/ha (nominal) in 200 L water/ha. A deionised water control and a toxic reference (11 mL/ha Perfekthion EC, equivalent to 4.35 g dimethoate in 200 L water/ha) were included in the study design.
Mortality was recorded on Days 1, 3, 7, 9, 11 and 14 after test initiation. Reproduction, measured as number of eggs and number of live and dead juvenile stages per female, was assessed on Days 7, 9, 11 and 14 after test initiation.

Findings:

Test formulation	**Control**	**Fosetyl-Al WG 80 (EXP 10369F)**		**Toxic standard**
Application	**Deionized water (200 L/ha)**	**18.75 kg/ha**	**12.0 kg/ha**	**11 mL Perfekthion EC/ha**
% mortality (1 week after the application	10	89.0	84.0	94.0
% corrected mortality	-	86.7	87.1	-
Reproduction rate (mean total # eggs per female)	9.0	0.0	0.3	0.7
Quotient of treated and untreated values (R)	-	0.00	0.03	-

Conclusions:
Under the worst-case conditions of this test, the LR_{50} of *Typhlodromus pyri* exposed to fresh dry deposits of Fosetyl-Al WG 80 (EXP 10369F) on glass plates was below 12.0 kg product/ha (9.6 kg a.s./ha), the lowest tested rate.

This study was already evaluated for the Annex I listing of fosetyl-Al.

- **RMS Conclusion: acceptable**

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: [redacted], S. *ET AL.* (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products in: Candolfi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 121 – 144.

Test endpoints according to current guideline:
- Cumulative juvenile mortality 7 days after treatment (day 7).
- Cumulative reproduction per females from day 7 to day 14.

Exposure according to current guideline:
Either glass plates, glass cells or glass discs are sprayed with the test item. 20 protonymphs (24 hours old) in each of 5 replicates are exposed to the dried spray residue for 14 days.

Evaluation according to current guideline:
Mortality assessment is carried out on day 3 (optional) and day 7 (obligatory). Reproduction per female is recorded 3 times from day 7 on, to day 14 with a maximum interval of 3 days. .

Validity Criteria:

	Guideline	**Test result**
Mortality rate	Mean mortality (dead + escaped) ≤ 20% at day 7	17%
Toxic reference mean mortality of protonymphs at day 7 (control corrected)	Between 50 and 100 %	94%
Reproduction (number of eggs per female in the control from day 7 to 14)	≥ 4	9.4

Study Remarks:
No major differences in the study design were found between the current guideline and the actual study. All procedures and assessments were performed as described in the guideline with the exception of the environmental conditions. Due to technical reasons, the relative humidity was less than 60% for up to 4 hours and temperature exceeded 27 °C for about 3 hours (max. 30.0 °C).

In the previous EU Annex I listing (Addendum to the DAR, 2009) it was concluded: “Both the standard and the extended laboratory study by [redacted] ([redacted].; 1999; M-184622-01-1 and [redacted]; 2000; M-238637-01-1) on *T pyri* revealed severe effects of EXP10369F at 15 kg a.s./ha to this predatory mite. [redacted] ([redacted]; 2001; M-202973-01-1), in contrast, found lower mortality, but significant impact on reproduction was observed. Both studies conducted by [redacted] are characterized by an extreme variability regarding the air humidity and/or temperature. These points are addressed as deviations in both studies. Although these measurements refer to the climatic chamber (in which the whole set-up was placed) and not to the test units, it cannot be excluded that the test organisms encountered these environmental variations. In the study performed by [redacted] the "island-method", a recent improvement of the test design has been applied. The "islands" are thin glass slides floating on a water surface, preventing mites from escape and leads to a very homogenous humidity. Therefore, the result achieved by [redacted] were more relevant.”

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline. The validity criteria of the current test guideline were fulfilled. Due to the observed deviations of air humidity and temperature the study is considered as less reliable compared to the study of [redacted] ([redacted]F; 2001; M-202973-01-1).

Report:	KCP 10.3.2.1/04 [redacted],; 2001; M-202973-01-1
Title:	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in the laboratory Aliette 80 WG (Aliette SAR+) Code: AE F053616 00 WG80 A101
Report No.:	C012583
Document No.:	M-202973-01-1
Guideline(s):	ESCORT 1994. Guidance document on regulatory testing procedures for pesticides with beneficial arthropods
Guideline deviation(s):	none
GLP/GEP:	**no**

Material and methods

Test item: Fosetyl-Al WG 80 (AE F053616 00 WG80 A101 or EXP 10369F, a.s. content 798 g/kg fosetyl-Al, batch no. OP990907).
Typhlodromus pyri, five replicates, each containing 20 protonymphs per treatment group, were exposed to fresh dried residues on glass plates at rates of 2.52, 8.81 and 18.87 kg test item/ha (nominal) in 200 L water/ha. These application rates correspond to 2, 7 and 15 kg a.s./ha, repectively. A tap water control and a toxic reference (3 g dimethoate in 200 L water/ha) were included in the study design. The "island method" adopted here is an open laboratory method for testing the effects of plant protection agents on *Typhlodromus pyri* in which the use of insect glue is not necessary (Joisten 2000).
Mortality was recorded at Days 3, 7, 10, 12 and 14 after test initiation. Reproduction, measured as number of eggs and number of live and dead juvenile stages per female, was assessed on Days 7 to day 14 after test initiation.

Findings

Up to day 7 of the test 91% of the mites survived in the control group, while 2% died, and 7% were missing. At 2.52, 8.81 and 18.87 kg test item/ha the survival rates at day 7 were 95%, 90% and 90%, respectively. There were no survivors at day 7 in replicates treated with the toxic reference substance.
The mean (± standard deviation) number of offspring produced per female in the control group was 11.3 (± 0.4). This compared to 8.8 (± 0.9) eggs/female at 2.52 kg test item/ha, 4.6 (± 1.9) at 8.81 kg test item/ha and 4.7 (± 0.5) eggs/female at 18.87 kg test item/ha. Due to 100% mortality no reproduction could be quantified in replicates with the toxic reference substance.

Corrected mortalities, reproduction rates and the % total effects are derived as follows:

Treatment	control	2.52 kg pr./ha	8.81 kg pr./ha	18.87 kg pr./ha	toxic standard
Mortality at day 7 [%]	9	5	10	10	100
Corrected mortality [%]	-	-4.4	1.1	1.1	100.0
Mean of total number of eggs/female	11.3	8.8**	4.6**	4.7**	-
Reduction of reproduction [%]	-	0.77	0.41	0.41	-
Total effect according to Overmeer & van Zon (1982) [%]	-	19.2	59.8	59.0	100.0

**: significantly different from the control with alpha < 0.05.

Conclusions

The effect of Fosetyl-Al WG 80 (Aliette WG, EXP10369F) to the predatory mite, *Typhlodromus pyri* under worst-case conditions (glass plate) was determined as follows: LR_{50} > 18.87 kg product/ha.

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: [redacted], S. *ET AL.* (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products in: Candolfi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 121 – 144.

Test endpoints according to current guideline:

- Cumulative juvenile mortality 7 days after treatment (day 7).
- Cumulative reproduction per females from day 7 to day 14.

Exposure according to current guideline:
Either glass plates, glass cells or glass discs are sprayed with the treatments. 20 protonymphs (24 hours old) in each of 5 replicates are exposed to the dried spray residue for 14 days.

Evaluation according to current guideline:
Mortality assessment is carried out on day 3 (optional) and day 7 (obligatory). Reproduction per female is recorded 3 times from day 7 on to day 14 with a maximum interval of 3 days.

Validity Criteria:

	Guideline	**Test result**
Mortality rate	Mean mortality (dead + escaped) ≤ 20% at day 7	9%
Toxic reference mean mortality of protonymphs at day 7 (control corrected)	Between 50 and 100 %	100%
Reproduction (number of eggs per female in the control from day 7 to 14)	≥ 4	11.3

Study Remarks:
No major differences were found between the current guideline and the actual study. All procedures and assessments were performed as described in the guideline. The reproduction formula used to calculate the effect on reproduction value was modified, deviating from the formula given in the current guideline, including the number of eggs that were counted on day 7. The influence on the result of the reproduction assessment is minor. Since on day 7, the highest number of eggs was observed in the control treatments a re-evaluation would slightly decrease the calculated effect on reproduction of the test item treatment groups.

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline. The validity criteria of the current test guideline were fulfilled.

Report: KCP 10.3.2.1/05[redacted]; 1999; M-184577-01-1
Title: Effects of EXP10369F on the carabid beetle Poecilus cupreus L. (Coleoptera, Carabidae) in the laboratory Final report
Report No.: R011795
Document No.: M-184577-01-1
Guideline(s): BBA: Part VI, 23-2.1.8, (1991)
US EPA OCSPP guideline number 850.SUPP
Guideline deviation(s): none
GLP/GEP: **yes**

Materials and methods:
Formulated product Fosetyl-Al WG 80 (EXP 10369F, a.s. content 794 g/kg fosetyl-Al, batch no. OP980953). *Poecilus cupreus* (5 replicates of each with 3 females and 3 males per treatment group) on quartz sand were sprayed over with 12.0 and 18.75 kg test item/ha (nominal) in 400 l water/ha. A tap water control and a toxic reference (Afugan 30 EC, 294 g pyrazophos in 400 L water/ha) were included in the study design.
Mortality was recorded 2 hours and 1, 2, 4, 7, 10 and 14 days after the application. Frozen fly pupae was provided as food at days 0, 2, 4, 7 and 10 at a rate of a pupa per viable beetle and the number of pupae remaining uneaten in each test unit was recorded on days 2, 4, 7, 10 and 14. Observations for symptoms of behaviour or physical abnormalities were recorded 2 hours and 1, 2, 4, 7, 10 and 14 days after applications. The endpoints measured in this study were mortality, food consumption and behaviour abnormalities.

Findings:

	Control	**Fosetyl-Al WG 80 (EXP10369F)**		**Toxic standard**
Application	**Water**	**12.0 kg pr./ha**	**18.75 kg pr./ha**	**Pyrazophos 294 g/ha**
Mortality [%]	3.3	0	0	100
Food Consumption [No. of fly pupae /beetle, cumulated over 14 days]	2.5	3.0	3.0	0.0

Observations:
None of the 30 beetles died after 14 days exposure to 12.0 and 18.75 kg test item/ha (nominal) in 400 L water/ha. In the control group one of the 30 beetles died by the end of the experiment. All 30 beetles (100%) died after application of 1 L/ha toxic standard Afugan 30 EC. No adverse effects of Fosetyl-Al WG 80 (EXP10369F) on food consumption of *Poecilus cupreus* occurred either in the group treated with 12.0 and 18.75 kg product/ha respectively.

Conclusions:
Under the conditions of this test, the No Observed Effect Rate (NOER) on *Poecilus cupreus* was in excess of 18.75 kg pr./ha.

☐ **RMS Conclusion: acceptable**

Further study information supplementing the original DAR summary:

Current Guideline:
[redacted], U., *et al.*, (2000) A method for testing effects of plant protection products on the carabid beetle *Poecilus cupreus* (Coleoptera, Carabidae) under laboratory and semi-field conditions in: Candfoldi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 87 – 106.

Test endpoints according to current guideline:

- Mortality and behavioural abnormalities
- Food uptake (number of fly pupae eaten per beetle)

Exposure according to current guideline:
Suitable test units are, for example plastic containers (outside dimensions: e.g. 18 x 13.5 cm, 6 cm high) filled with 250 ± 1 g dry quartz sand (particle size 0.1 to 0.4 mm, at least 99% Si-oxide). Test vessels made of glass or metal can also be used; the surface area of the substrate must have a sieve of 180 ± 20 cm^2 and the vessel depth 6 ± 1 cm. The quantity of sand must be adapted to the size of the vessel (filling height approx. 1 cm). Transparent lids should allow air ventilation. Before the animals are put into the test units, the dry sand in the test is evenly wetted with 45 ± 4 mL deionised water without disturbing the sand surface. The beetles have to be exposed to the test conditions at least for 3 days before the application. After application of the test item to the beetles in the test units, they are observed for at least 14 days.

Evaluation according to current guideline:
Mortality and behavioral abnormalities are assessed 1 to 3 hours and 1, 2, 4, 7, 10 (or 11) and 14 days after application.
Food consumption is assessed by the number of fly pupae consumed or untouched at day 2, 4, 7, 10 (or 11) and 14 after applications.

Validity Criteria:

	Guideline	Test result
Maximum mortality after 2 weeks in the control	≤ 2 beetles (6.7%)	1 beetle (3.3%)
Mortality rate in the reference treatment after 2 weeks (control corrected)	65 ± 35%	100 %

Study Remarks:
No major differences were found between the current guideline and the actual study. The temperature raised by 0.5 °C above the agreed limit (22 °C) for a period of 9.5 hours, but this minor increase didn't had an influence on the 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.

Report:	KCP 10.3.2.1/06[redacted]; 2000; M-238636-01-1
Title:	Effects of EXP10369F on the Reproduction of Rove Beetles Aleochara bilineata Gyll. (Coleoptera, Staphi\ylinidae) in the Laboratory
Report No.:	B002978
Document No.:	M-238636-01-1
Guideline(s):	IOBC: Moreth & Naton (1992)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Materials and Methods:
Formulated product Fosetyl-Al WG 80 (EXP 10369F, a.s. content 808 g/kg fosetyl-Al, batch no. OP990544). *Aleochara bilineata* (10 female and 10 male beetles per replicate, 4 replicates per treatment group) were exposed immediately after spraying to spray deposits on quartz sand treated with 12.0 and 18.75 kg test item/ha (nominal) in 400 L water/ha. A tap water control and a toxic reference (1100 mL Perfekthion EC in 400 L water/ha) were included in the study design.
Once a week approx. 500 *Delia antiqua* pupae per container were added (at day 7, 14 and 21, 28 days after application the pupae were washed out of the sand and transferred into a separate emergence container).
Emergence commenced approx. 4 weeks after treatment and continued for further 4 weeks until emergence of the new generation of beetles had finished (F1; beetles hatched from eggs laid in the sand from female test beetles during the exposure time and developed in the offered fly pupae) of beetles had finished. Emerging beetles were counted and removed from the emergence containers at least 3 times per week; following 3 consecutive days of no beetle emergence it was assumed that no further emergence would occur and the assessment was finished.

Findings:

Application	**Untreated control (Water (400 L/ha)**	**Fosetyl-Al WG 80 (EXP10369F)**		**Toxic standard 435.3 g dimethoate/ha**
		18.75 kg pr./ha	**12.0 kg pr./ha**	
Mean number of emerged beetles per replicate	801	751	795	173
Reduction of reproduction efficiency (%)	-	14.8	9.8	80.4
Significant compared to the control (Dunnett test, alpha = 0.05)	-	significant	not significant	significant

Conclusions:
Under the conditions of this test, the no-effect rate for rove beetles *Aleochara bilineata* was 12.0 kg product/ha. The rate with mortality and effects on reproduction below 50% was in excess of 18.75 kg product/ha.

This study was already evaluated for the Annex listing of fosetyl-Al.

- **RMS Conclusion: acceptable**

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: [redacted] C., *ET AL.* (2000). A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions in: Candfoldi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 1 – 12

Test endpoints according to current guideline:

- Reproductive efficiency: total number of beetles emerged from the offered fly pupae

Exposure according to current guideline:
The test units used do assess beetle fecundity consists of a container (e.g. a glass or plastic dish or cylinder) with a minimum ground surface of 150 cm^2 with a layer of moist quartz sand (laboratory test) or sandy soil (extender laboratory test) as substrate. The test unit is covered with a lid with an opening that is covered with fine mesh nylon netting. The layer of substrate in the container is at least 4 cm deep (minimum volume 600 cm^3). For the standard laboratory test, quartz sand is used as inert substrate. The particle size should be within the range of 0.4 to 0.8 mm. Before introducing the test organisms into the test units, the sand is moistened with tap water at a ratio sand:water of approximately 10:1 vol./vol.. Ten pairs of male and female adult beetles, between one and seven days old, are then introduced after the application of the test item. A total of approximately 1500 onion fly *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) pupae are added to the test substrate during the following three weeks as hosts for the beetle larvae. After removing the pupae from the substrate, they are placed into hatching test units. The purpose of the hatching units is to reliably catch all adults *Aleochara bilineata* hatching from the onion fly pupae.

Evaluation according to current guideline:
The reproductive efficiency of *Aleochara bilineata* is assessed as the number of beetles of the Fl generation emerged from the offered fly pupae.

Validity Criteria:

	Guideline	**Test result**
Average number of beetles emerging from the fly pupae in the control	> 400 (>26.7%)	881 (58.7%)
Minimum reduction of reproductive capacity in the reference item treatment (control corrected)	50%	80.4%

Study Remarks:
No major differences were found between the current guideline and the actual study. Some minor modifications compared to the guideline were applied to the study protocol. These small improvements (e.g. the height of the glass beakers) had no effect on the outcome of the study and it made it easier to conduct the study.

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline. The validity criteria of the current test guideline were fulfilled.

Report: KCP 10.3.2.1/07[redacted]; 1999; M-184632-01-1
Title: Effects of EXP10369F on the ladybird beetle Coccinella septempunctata L. (Coleoptera, Coccinellidae) in the laboratory - Final report
Report No.: R011815
Document No.: M-184632-01-1
Guideline(s): BBA: Part VI, No.23-2.1.5
Guideline deviation(s): none
GLP/GEP: **yes**

Materials and methods:
Formulated product Fosetyl-Al WG 80 (EXP 10369F; a.s. content 808 g/kg fosetyl-Al; batch no. OP990544).
4 to 5 days old *Coccinella septempunctata* larvae (50 larvae, one larvae per test unit) were exposed for approx. 10 to 22 days to fresh dried spray deposits of 12.0 and 18.75 kg product/ha (nominal) on glass plates. A tap water control and a toxic reference (10 mL/ha Afugan 30 EC, equivalent to 2.95 g pyrazophos/ha) were included in the study design. The number of living and dead larvae, pupae and adults was counted at least daily during the weekly working days. Living adults from each experimental groups were sexed and transferred to separate insect-rearing cages. The pre-oviposition period lasted 6 to 14 days and the oviposition period 3 weeks. The endpoints assessed in this study were pre-imaginal mortality (living and dead larvae and pupae during the exposure period) and effects on reproduction (measured as the number of eggs produced during the second and third week of oviposition and number of larvae hatched from eggs laid in the second week of oviposition).

Findings:

Test substance	**Control**	**Fosetyl-Al WG 80 (EXP10369F)**		**Toxic standard**
Application	**Water**	**12.0 kg pr./ha**	**18.75 kg pr./ha**	**Pyrazophos 2.95 g/ha**
Mortality [%]	16.0	14.0	82.0	68.0
Corrected mortality [%]	-	2.4*	78.6	61.9
Reproduction (No. of fertile eggs/female/day, mean ± SD)	12.2 ± 5.2	9.8 ± 3.8 ns	6.2 ± 6.9 ns	7.6 ± 6.5 ns

*: the negative value reflects the lower mortality than in the control
ns: differences not statistically significant

During the exposure period, 5 larvae and 3 pupae of the 50 larvae (16%) died in the control group and 33 larvae and 1 pupa of the 50 larvae (68%) died in the toxic standard group. 7 larvae of the 50 exposed larvae (14.0%, M_{corr} 2.4%) died during exposure in the group treated with 12.0 kg product/ha and 41 larvae and 1 pupa of the 50 larvae (82.0%, M_{corr} 78.6%) in the group treated with 18.75 kg product/ha of Fosetyl-Al WG 80.
Surviving *C. septempunctata* produced 9.8 fertile eggs per female per day in the group treated with 11.88 kg/ha of EXP10369F and 19.2 fertile eggs per female per day in the group treated with 18.56 kg/ha of EXP 10369F. In the control group surviving *C. septempunctata* produced 12.2 fertile eggs per female per day. There were no statistically significant differences between the test substance groups and the control.

Conclusions:
Under the conditions of this test, spray treatments of Fosetyl-Al WG 80 (EXP10369F) at a rate equivalent to 12 kg product/ha (nominal) did not cause adverse lethal or sub-lethal effects on ladybird beetles (No observed effect rate). Thus 18.75 > LR_{50} > 12.0 kg product/ha.

This study was already evaluated for the Annex I listing of fosetyl-Al.

❑ **RMS Conclusion: acceptable**

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: [redacted] *et al.,* (2000) A laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). In Candolfi, *et al.,* (eds.) (2000) Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC/WPRS, Gent, pp 45-56.

Test endpoints according to current guideline:

- Pre-imaginal mortality (pupation after 10 to 15 days).
- Reproductive performance of the ecdysed beetles over a 2 week period.

Exposure according to current guideline:
Glass plates are sprayed with the compound to be tested. After spray deposits have dried, 3 to 5 days old larvae (n=40) are individually confined on these glass plates until they enter the pupal stage. After ecdysis (approx. 1.15 days after study initiation) beetles are removed and transferred to non-treated breeding cages. The eggs laid are sampled over a period of two weeks and observed for fertility (larval hatch).

Evaluation according to current guideline:
The number of ecdysed beetles is recorded daily for each treatment (control, test, reference group) separately. If more than 50% of the larvae exposed to the test item survive and can successfully ecdysed, the reproductive performance of the beetles is assessed. After control beetles have started to lay eggs all surviving beetles of the test item and the control group are taken and confined in breeding containers. The number of eggs laid per viable female in the test item and the control group is recorded daily over two weeks.

Validity Criteria:

	Guideline	**Test result**
Average pre-imaginal mortality on the control	≤30%	16 %
Pre-imaginal mortality in the reference treatment	≥40%	68 %
Number of eggs/female/day on the control	> 2	16.5

Study Remarks:
No differences were found between the current guideline and the actual study. All procedures and assessments were performed as described in the guideline. Slight differences occurred some times during the study, with the temperature rising or decreasing by 1 or 2 °C by 2 to 6 hours. However this small difference didn't lead to any effect on the study. Likewise, similar differences were found in the humidity values for some hours during the study, nevertheless these changes also had no effect on the study results. Some corrections were also made for the larval hatch. Instead of separating eggs only during the 2nd week, in this study, eggs were separated also on the 3rd week. Due to the high abundance of eggs (2000 when only 200 are needed), this had no effect on the larval hatch. All study parameters and assessments were then obtained as described in the guideline.

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline. The validity criteria of the current test guideline were fulfilled.

Report: KCP 10.3.2.1/08[redacted]P; 1999; M-184635-01-1
Title: Effects of EXP10369F on the carabid beetle Poecilus cupreus L. (Coleoptera, Carabidae) in the laboratory - Non-GLP-screening test
Report No.: R011817
Document No.: M-184635-01-1
Guideline(s): BBA: Part VI, 23-2.1.8, (1991)
US EPA OCSPP 850.SUPP
Guideline deviation(s): none
GLP/GEP: **no**

Non-GLP screening study, not evaluated from RMS in Annex 1 listing of Fosetyl-Al.

Materials and methods:
Fosetyl-Al WG 80, EXP10369F, (purity: fosetyl-Al 808 g/kg), specification Lot no.: QP990544); under laboratory conditions *Poecilus cupreus* (18 beetles per treatment group) were sprayed with 124 kg EXP10369F/ha in 400 L water / ha (corresponding to 100 kg a.s./ha).
The control animals were sprayed with tap water. Additionally 1 L Afugan EC 30 in 400 L water/ha was used as a toxic standard.
Mortality and behavioural abnormalities were recorded 2 hours and 1, 2, 4, 7, 10 and 14 days after the application. Frozen fly pupae was provided as food at day 0, 2, 4, 7 and 10 in a rate of a pupa per viable beetle and the number of pupae remaining uneaten in each test unit was recorded on days 2, 4, 7, 10 and 14. Endpoints were mortality, behavioural impairment and food consumption of the survivors.

Findings:

Test substance	**EXP10369F**		
Test species	**Carabid Beetle *Poecilus cupreus***		
Exposure	Application onto the soil of the trays and the beetles		
Test formulation	Control	EXP10369F	Toxic Standard
Application	water 400L/ha	124 g EXP10369F/ha (100 kg a.s./ha)	1 L Afugan/ha
Mortality [%]	0.0	0.0	77.8
Food Consumption [No. of fly pupae /beetle, cumulated over 2 weeks]	3.2	4.3	3.9

Observations:
None of the 18 beetles died after a 14 days exposure to exposure of 124 kg EXP10369F/ha (100 kg fosetyl-Al/ha). In the control group none of the 18 beetles died by the end of the experiment. 14 of 18 beetles (77.8%) died after application of 1 L/ha toxic standard Afugan 30 EC. No adverse effect of EXP10369F on food consumption or behavioural abnormalities on beetles *Poecilus cupreus* occurred at a rate of 124 kg EXP10369F/ha (100 kg fosetyl-Al/ha).

Conclusions:
EXP10369F applied at 124 kg/ha (100 kg fosetyl-Al/ha) is harmless to carabid beetles *Poecilus cupreus*.

Further study information supplementing the original DAR summary:

Current Guideline:
[redacted] U., *et al.* (2000) A method for testing effects of plant protection products on the carabid beetle *Poecilus cupreus* (Coleoptera, Carabidae) under laboratory and semi-field conditions in: Candfield, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 87 – 106.

Test endpoints according to current guideline:

- Mortality and behavioural abnormalities
- Food uptake (number of fly pupae eaten per beetle)

Exposure according to current guideline:
Suitable test units are, for example plastic containers (outside dimensions: e.g. 18 x 13.5 cm, 6 cm high) filled with 250 ± 1 g dry quartz sand (particle size 0.1 to 0.4 mm, at least 99% Si-oxide). Test vessels made of glass or metal can also be used; the surface area of the substrate must have a surface of 180 ± 20 cm^2 and the vessel depth 6 ± 1 cm. The quantity of sand must be adapted to the size of the vessel (filling height approx. 1 cm). Transparent lids should allow air ventilation. Before the animals are put into the test units, the dry sand in the test is evenly wetted with 45 ± 1 mL deionised water without disturbing the sand surface. The beetles have to be exposed to the test conditions at least for 3 days before the application. After application of the test item to the beetles in the test units, they are observed for at least 14 days.

Evaluation according to current guideline:
Mortality and behavioral abnormalities are assessed 1 to 3 hours and 1, 2, 4, 7, 10 (or 11) and 14 days after application.
Food consumption is assessed by the number of fly pupae consumed or untouched at day 2, 4, 7, 10 (or 11) and 14 after applications.

Validity Criteria:

	Guideline	Test result
Maximum mortality after 2 weeks in the control	2 beetles (6.7%)	0 beetles (0%)
Mortality rate in the reference treatment after 2 weeks (control corrected)	65 ± 35%	77.8%

Study Remarks:
No major differences were found between the current guideline and the actual study. When comparing to the guideline, in the present study, the number of beetles used per treatment was significantly lower (18 compared to 30). Nevertheless, it should be considered that in this specific study, there was no mortality in the control. Therefore, the study would have identified a mortality of 30% as statistically significant based on Fisher's exact test. Since there was no mortality observed in the test item group, it can be concluded that the total number of individuals is high enough to meet the guideline requirements.

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.

Report:	KCP 10.3.2.1/09[redacted],; 1999; M-184609-01-1
Title:	Effects of EXP10369F on the wolf spider Pardosa spec. (Araneae, Lycosidae) in the laboratory - Non-GLP screening test
Report No.:	R011805
Document No.:	M-184609-01-1
Guideline(s):	BBA: Draft Guideline 1994 USA EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**no**

Non-GLP screening study, not evaluated from RMS in Annex I listing of fosetyl

Materials and methods:
Fosetyl-Al WG 80, EXP10369F, (purity: fosetyl-Al 794 g/kg), specification Lot no.: QP98090); under laboratory conditions *Pardosa spec.* (20 spiders per treatment group, 10 females and 10 males) and sand were sprayed with 126 kg EXP10369F/ha in 400 L water/ha (corresponding to 100 kg a.s./ha).
The control was sprayed with tap water.
Mortality and behavioural abnormalities were recorded 2 hours and 1, 2, 7 and 14 days after the application. Frozen adult flies were provided as food ad libitum.

Findings:

Experimental time	Test substance		Water treated control	
	Mortality [%]	Behavioural abnormalities [%]	Mortality [%]	Behavioural abnormalities [%]
2 hours	0.0	0.0	0.0	0.0
day 1	0.0	0.0	0.0	0.0
day 2	0.0	0.0	0.0	0.0
day 7	25.0	0.0	0.0	0.0
day 14	65.0	5.0	0.0	0.0

Observations:
1 female spider was apathetic on day 7 and 1 male spider was lying on the back on day 14.

Conclusions:
13 of the 20 spiders (65%) died by the end of the experiment after 14 days of exposure to 126 kg EXP10369F/ha (100 kg fosetyl-Al/ha). In the control group none of the 20 spiders died by the end of the experiment.

The reference toxic standard treated with 4 ml/l a Thiodan did result in 35% mortality after 14 days. The experiment was performed in parallel to this experiment.

Further study information supplementing the original DAR summary:

Current Guideline:
[redacted], I. *et al.* (2000) A method for testing effects of plant protection products on spiders of the genus *Pardosa* (Araneae, Lycosidae) under laboratory conditions in: Candfoldi, M.P. *Et al.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 71-86.

Test endpoints according to current guideline:
- Mortality and behavioural impacts
- Food uptake

Exposure according to current guideline:
Test units are, for example, plastic containers (e.g. outside dimensions. 11.5 x 11.5 cm, 6 cm high) filled with 125 ± 1 g dry quartz sand (particle size 0.1 to 0.4 mm, at least 99% Si-oxide). Test units made of glass or metal may also be used, but the surface area of the substrate should be 90 ± 20 cm^2 and the unit depth 6 ± 1 cm. The quantity of sand must be adapted to the size of the vessel (filling depth of approx. 1 cm sand). Transparent lids should allow air ventilation. Before the animals are placed into the test units, the sand is evenly wetted with distilled or deionised water so that it is at 70 ± 5% of its pre-determined maximum water-holding capacity. The water should be added without disturbing the sand surface. The animals are subsequently placed into the units.
If the test and reference item is to be applied with a sprayer, it will be diluted just before treatment application in deionised or distilled water for application at a rate of 400 L/ha. After the application of the treatments to the spiders in the test units, they are observed for at least 14 days. If effects still occur in the second week after application, the test should be prolonged for another week.

Evaluation according to current guideline:
The test units are inspected after 1 to 3 hours and 1, 2, 3, 4, 7, 10 and 14 days after treatment application. If effects in the group treated with the test item, already occur 1 to 3 hours after application, a further assessment must be carried out approximately 2 hours after the first assessment. On each occasion, effects on the test animals should be recorded, as should the number of live and dead spiders. Furthermore, all skins found, all females which have built cocoons and the number of animals sitting on the underside of the lid (thus escaping contact with the test item) should be noted.

Validity Criteria:

	Guideline	**Test result**
Maximum mortality after 2 weeks in the control	≤6.7%	0.0%
Mortality rate in the reference treatment after 2 weeks (control corrected)	65 ± 35%	35%

Study Remarks:
No major differences were found between the current guideline and the actual study. The toxic reference treatment is not presented in the study but was performed in parallel. The results from this reference treatment match the validity criteria, and therefore can be considered for the study.

Conclusion:
The study was not conducted under GLP. 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.

Supplemental information from the literature

Report:	KCP 10.3.2.1/10[redacted];[redacted];[redacted]; 2014; M-484397-01-1
Title:	Side effects of different pesticides used in citrus on the adult stage of the parasitoid Aphytis melinus DeBach (Hymenoptera Aphelinidae) and its progeny.
Report No.:	M-484397-01-1
Document No.:	M-484397-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Executive Summary

Fosetyl-aluminium (fosetyl-Al) was tested on the adult females of *Aphytis melinus* to determine the effects on parasitoid survival and fecundity. Survival and fecundity were not significantly affected. Also the test item had a low value of reduction of beneficial capacity. According to the IOBC criteria it was classified as slightly harmful.

Material and Methods

A. Material

1. Test material

Test item:	Pomoral®-WP
Active substance(s):	Fosetyl-Al (80%)
Chemical state and description:	Not reported
Source of test item:	[redacted]
Lot/Batch number:	Not reported
Purity:	Not reported

2. Test organism(s)

Species:	*Aphytis melinus*
Host:	*A. nerii*
Source of test species:	[redacted]

B. Study design and methods

1. Test procedure

Test system (study type):	Laboratory Petri dish assay with fresh residues
Conduction:	Pesticides were left to dry at room temperature for an hour, then adult females of *A. melinus* (24-48 h old) were introduced into, adding several drops of honey as food. Parasitoid mortality on each Petri dish was evaluated after 24 h. Surviving females were transferred to a correspondent ventilated container with a piece of squash with drops of honey and an excess of *A. nerii* scales. Females were left with the scales for 48-72 h to parasitize the hosts, and the resulting parasitoid offspring were counted and sexed following their emergence.
Application technique:	Test item was applied to tops and bottoms of Petri dishes (5 cm diameter) with a potter precision spray tower, 0.7 bar pressure, calibrated to leave 1.5 mg of solution cm^{-2} [150 L/ha].
Application rate:	19.2×10^3 ppm a.i.
Number of replicates:	4-6
Individuals per replicate:	6-8
Test conditions:	The experiment was carried out at 25 ± 1 °C, a 16:8 L:D photoperiod and 60 ± 5% relative humidity.

2. Observations and measurements:

Biological parameters measured: Contact toxicity to adult parasitoids; effects on parasitization activity and sex ratio of parasitoid offspring.

Statistical analyses: Data on adult parasitoid mortality, the mean number of offspring produced per surviving parasitoid, and the sex ratio of the offspring (as % female) were subjected to one-way ANOVA using the results of each Petri dish as replicate. Means were separated using Tukey's honest significant difference test when analysis of variance were significant at $p < 0.05$. All data needing to be normalized were transformed before being analyzed.

Results

1. Validity criteria:
No validity criteria defined.

2. Biological findings:
Table 1 shows the mortality and the reduction of beneficial capacity.

Table 1: Mortality of adults of *Aphytis melinus* (corrected with the Abbott's formula) and reduction of beneficial capacity (RBC) indices

	Corrected mortality (%)(mean ± se)	RBC[a] (%)(mean ± se)	IOBC
Fosetyl-Al	3.3 ± 3.3	31.7 ± 26.3	2

[a] Reduction of Beneficial Capacity. Toxicity effects grouped into four categories according to IOBC: slightly harmful (30-79%).

Fosetyl-Al produced a mortality of 3.3% which did not differ from the control. The fecundity was not significantly affected. The test item had a low value of reduction of beneficial capacity.

Conclusion
According to the IOBC criteria it was classified as slightly harmful.

Comment by the Notifier
The study results are in line with the available regulatory data for the parasitoid *Aphidius rhopalosiphi*. The data are considered as supplemental information.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Report:	KCP 10.3.2.2/01 [redacted],; 2000; M-238637-01-1
Title:	Effects of EXP10369F on the Predatory Mite Typhlodromus pyri Scheuten (Acari; Phytoseiidae) - Extended Laboratory Study
Report No.:	B002979
Document No.:	M-238637-01-1
Guideline(s):	IOBC: Louis & Ufer (1995), Overmeer (1988)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Materials and Methods:
Formulated product Fosetyl-Al WG 80 (EXP 10369F, a.s. content 7808 g/kg fosetyl-Al, batch no. OP990544).
Typhlodromus pyri, 5 replicates, each containing 50 protonymphs per treatment group, were exposed to fresh dried residues on detached primary leaves of the dwarf bean *Phaseolus vulgaris* at rates of 7.5 and 18.75 kg test item/ha (nominal) in 200 L water/ha. A deionised water control and a toxic reference (40 mL Perfekthion EC, equivalent to 35.8 g dimethoate in 200 L water/ha) were included in the study design. Mortality was recorded on days 3, 7, 11 and 14. Assessments of egg production and number of juveniles (reproduction endpoint) were conducted on days 7, 9, 11 and 14.

Findings:

Test formulation	**Control**	**Fosetyl-Al WG 80 (EXP10369F)**		**Toxic standard**
Application (200 L/ha)	**deionised water)**	**7.5 kg/ha**	**18.75 kg/ha**	**35.8 g dimethoate/ha**
Mortality (1 week after last application) [%]	12.0* ± 8.4	73.0* ± 25.4	99.0* ± 2.2	94.0* ± 6.5
Corrected mortality [%]		69.3	98.9	93.2
Reproduction rate (mean total # eggs per female)	10.2 ± 0.5	2.3* ± 1.5	Not evaluated	Not evaluated
Reduction of reproduction [%]	-	77.5	-	-

* Significance compared to the control (Bonferroni U-test, alpha = 0.05)

After 1 week exposure to residues of Fosetyl-Al WG 80, 58 mites died and 15 mites escaped making a total mortality of 73% in the group treated with 7.5 kg/ha and 91 mites died and 8 mites escaped making a total mortality of 99% in the group treated with 18.75 kg/ha; in the water treated control group 3 mites died and 9 escaped (total mortality of 12%), 62 mites were dead and 32 escaped (94% mortality) after 1 week of exposure in the toxic standard group.
The mean reproduction rate of the mites in the group treated with 7.43 kg test item/ha was 2.3 eggs per female compared to 10.2 eggs per female in the control group. The reproduction in the group treated with 18.56 kg test item/ha and in the toxic standard group was not evaluated due to 99 and 94% mortality, respectively.

Conclusions:
Under the conditions of this test, the LR_{50} of *Typhlodromus pyri* exposed to fresh dried residues on detached bean leaves was below 7.5 kg product/ha.

This study was already evaluated for the Annex I listing of fosetyl-Al.

- **RMS Conclusion: acceptable**

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: [redacted], S. *ET AL.* (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products in: Candolfi, M.P. *ET AL.* (eds.). - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 121 – 144.

Test endpoints according to current guideline:

- Cumulative juvenile mortality 7 days after treatment (day 7).
- Cumulative reproduction per females from day 7 to day 14.

Exposure according to current guideline:
Either glass plates, glass cells or glass discs are sprayed with the treatments. 20 protonymphs (24 hours old) in each of 5 replicates are exposed to the dried spray residue for 14 days.

Evaluation according to current guideline:
Mortality assessment is carried out on day 3 (optional) and day 7 (obligatory). Reproduction per female is recorded 3 times from day 7 on to day 14 with a maximum interval of 3 days.

Validity Criteria:

	Guideline	**Test result**
Mortality rate	Mean mortality (dead + escaped) ≤ 20% at day 7	12%
Toxic reference mean mortality of protonymphs at day 7 (control corrected)	Between 50 and 100 %	94%
Reproduction (number of eggs per female in the control from day 7 to 14)	≥ 4	10.2

Study Remarks:
The design used in this study is aligned with the recommendations of the current guideline. As recommended by the guideline, when dealing with extended laboratory tests for this insect species, the glass plates should be replaced by leaf material, as happened in the present study.
Instead of exposing the insects to the dried residues of the test item exactly at the same time, in this study the specimens were subjected with different timings after application. Due to technical reasons, the relative humidity was less than 60% for approximately 19 hours in total on day 3 and 4 after application.
In the previous EU Annex I listing (Addendum to the DAR, 2009) it was concluded: “Both the standard and the extended laboratory study by [redacted] ([redacted].; 1999; M-184622-01-1 and [redacted]; 2000; M-238637-01-1) on *T. pyri* revealed severe effects of EXP10369F at 15 kg a.s./ha to this predatory mite. [redacted] ([redacted]; 2001; M-202973-01-1), in contrast, found lower mortality, but significant impact on reproduction was observed. Both studies conducted by [redacted] are characterized by an extreme variability regarding the air humidity and/or temperature. These points are addressed as deviations in both studies. Although these measurements refer to the climatic chamber (in which the whole set-up was placed) and not to the test units, it cannot be excluded that the test organisms encountered these environmental variations. In the study performed by [redacted] the "island-method", a recent improvement of the test design has been applied. The "islands" are thin glass slides floating on a water surface, preventing mites from escape and leads to a very homogenous humidity. Therefore, the result achieved by [redacted] were more relevant.”

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline. The validity criteria of the current test guideline were fulfilled. Due to the observed deviations of air humidity the study is considered as less reliable compared to the study of [redacted] ([redacted]; 2001; M-202973-01-1).

Report:	KCP 10.3.2.2/02[redacted]; 2007; M-295474-01-1
Title:	Effects of Fosetyl-Al WG 80 on the predatory mite Typhlodromus pyri, extended laboratory study - dose response test
Report No.:	37191062
Document No.:	M-295474-01-1
Guideline(s):	[redacted] et al., 2000: Laboratory residual contact test with the predatory mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products Oomen 1988: Guideline for the evaluation of side-effects of pesticides on Phytoseiulus persimilis A.-H. US EPA OCSPP 840.3UPP
Guideline deviation(s):	Due to the slow development at 3 dose rates (5.0, 11.25 and 20.0 kg product/ha) the sex ratio for the reproduction assessment was balanced on day 10 instead of day 7. Due to the same reason and due to the lack of a sufficient number of males in some replicates of 2 dose rates (20.0 and 40.0 kg product/ha) the demanded sex ratio of 1 male : 5 females could not be reached.
GLP/GEP:	**yes**

Material and methods
Test item: Fosetyl-Al WG 80 (a.s. content 80 %g/kg, fosetyl-Al; TOX 07504-00; workorder: 06008273, batch ID.: 2006-00355, supplier batch ID: EV3000006, material nr.: 05925589, specification no.: 102000001579).
Typhlodromus pyri six replicates, each containing 10 protonymphs per treatment group, were exposed to fresh dried residues on detached bean leaves at rates of 2.5, 5.0, 11.25, 20.0, 40.0 and 80.0 kg test item/ha in 200 L water/ha. A deionised water control and a toxic reference (40 mL Perfekthion EC, equivalent to 15.8 g dimethoate in 200 L water/ha) were included in the study design.
Mortality was recorded on days 3 and after application, 9, 11 and 14. Assessments of egg production and number of juveniles (reproduction endpoint) were conducted at 3 assessment days within one week.

Findings

Treatment	Mortality [a] [%]		Corrected mortality [b] [%]	Reproduction [c] eggs/ female		Reduction of reproduction [d] [%]
Control	18.3		-	5.1		-
2.5 kg product /ha	16.7	n.s.	-2.0	4.7	n.s.	7.9
5.0 kg product /ha	35.0	n.s.	20.4	2.2	n.s.	57.6
11.25 kg product /ha	11.7	n.s.	-8.2	4.5	n.s.	11.2
20.0 kg product /ha	16.7	n.s.	-2.0	2.9	n.s.	43.8
40.0 kg product /ha	11.7	n.s.	-8.2	3.4	n.s.	32.3
80.0 kg product /ha	15.0	n.s.	-4.1	6.1	n.s.	-20.2
40 mL Perfekthion/ha (Reference Item)	100.0	*	100.0	n.a.		-
LR_{50}	> 80.0 kg product/ha					
ER_{50}	> 80.0 kg product/ha					

[a] n.s. = not significant, * = significant; Fisher Exact Test, $\alpha = 0.05$
[b] Negative value means lower mortality compared to the control
[c] n.s. = not significant; Dunnett-Test, $\alpha = 0.05$
[d] Negative value means increased reproduction compared to the control
n.a. = not applicable

Conclusions

Under extended laboratory conditions the LR_{50} of Fosetyl-Al WG 80 is estimated to be greater than 80.0 kg product/ha.
The reproductive capacity of *T. pyri* was tested at 2.5, 5.0, 11.25, 20.0, 40.0 and 80.0 kg product/ha. There was no statistically significant effect on reproduction up to and including 80.0 kg product/ha compared to the control.
At 5.0 kg product/ha the effect on reproduction was above the trigger value of 50% (57.6%) and the mites were underdeveloped, but at all higher dose rates the effect was below the trigger value 50% and less mites were underdeveloped. Therefore it can be assumed that the high effect at 5.0 kg product/ha was not test item related. The ER_{50} is > 80 kg product/ha.

Further study information supplementing the original DAR summary:

Current Guideline:
Reference: [redacted], S. *ET AL.* (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products in: Candolfi, M.P. *ET AL.* (eds.) - Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/ WPRS publication, 2000, 121 – 144.

Test endpoints according to current guideline:

- Cumulative juvenile mortality 7 days after treatment (day 7).
- Cumulative reproduction per females from day 7 to day 14.

Exposure according to current guideline:
Either glass plates, glass cells or glass discs are sprayed with the treatments. 20 protonymphs (24 hours old) in each of 5 replicates are exposed to the dried spray residue for 14 days.

Evaluation according to current guideline:
Mortality assessment is carried out on day 3 (optional) and day 7 (obligatory). Reproduction per female is recorded 3 times from day 7 on, to day 14 with a maximum interval of 3 days.

Validity Criteria:

	Guideline	Test result
Mortality rate	Mean mortality (dead + escaped) ≤ 20% at day 7	18.3%
Toxic reference mean mortality of protonymphs at day 7 (control corrected)	Between 50 and 100 %	100%
Reproduction (number of eggs per female in the control from day 7 to 14)	≥ 4	5.1

Study Remarks:
The actual study follows the recommendations of the guideline. As recommended by the guideline, when dealing with extended laboratory tests for non-target arthropods, the glass plates were replaced by leaf material. The reduced number of specimens per replicates used in the present study (10 instead of 20) is not a guideline deviation since 6 replicates have been used and the guideline states that for dose responses testing fewer replicates are needed. The current guideline is referring to the recommendations of Grimm *et al.,* (2001) in which the number of specimens per replicate is the same (10) as in the present study.

Conclusion:
The test design of the actual study is in line with the recommendations of the current guideline. The validity criteria of the current test guideline were fulfilled.

Report:	KCP 10.3.2.2/03 [redacted],; 2011; M-412084-01-1
Title:	Dose-response toxicity (LR50) of Fosetyl-AL WG 80% w/w to the ladybird Coccinella septempunctata L. under extended laboratory conditions
Report No.:	11 10 48 020 A
Document No.:	M-412084-01-1
Guideline(s):	IOBC Guideline (SCHMUCK et al. 2000), modified; use of natural substrate (detached bean leaves) instead of glass plates (extended laboratory test) US EPA OCSSP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

Materials and Methods:
The effects of the test item Fosetyl-Al WG 80% w/w [analysed active ingredient: 77.7% w/w Fosetyl-aluminium (LS 747831), Specification No.: 102000001579-03, Batch No.: EV38000066, Sample description: FAR01374-01, Material No.: 05921589] were tested under extended laboratory conditions after contact exposure of larvae of the ladybird *Coccinella septempunctata* L. to dried spray residues of the test item with rates of 9, 15, 26, 45 and 64 kg a.s./ha (equivalent to 12, 19, 33, 58 and 82 kg product/ha, based on analysed content of a.s.) in 200 L deionised water/ha applied on bean leaves. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (30 mL product/ha, nominally equivalent to 12 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item. Larvae of *Coccinella septempunctata* L. were exposed to the residues in 40 replicates per treatment group with 1 larva per replicate in the test item, reference item and control treatments, respectively. During the assessments, the larvae were fed with black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris). The number of dead larvae and pupae and hatched beetles as well as the number of eggs laid and larvae hatched (F1) were recorded over a period of 49 days. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated.

All validity criteria according to [redacted] *et al.* (2000) for conducting the laboratory test with *Coccinella septempunctata* were met.

Findings:

Test item	**Fosetyl-Al WG 80% w/w**			
Test object	*Coccinella septempunctata* L.			
Exposure	Dried spray deposits on detached bean leaves			
Treatment	**Mortality** [2]	**Reproduction**		
		Fecundity	Fertility	
	after 18 days	average number of eggs/ viable female/day	mean hatching rate	average number of fertile eggs/ viable female/day
	[%]	(number)	[%]	(number)
Control	22.5	4.1	76.3	3.1
Application rate [1] [kg a.s./ha]	Corrected mortality [3] [%]			
9	6.5 (n.s.)	4.5	76.8	3.5
15	3.2 (n.s.)	3.8	76.2	2.8
26	3.2 (n.s.)	4.1	76.1	3.1
45	-3.2 (n.s.)	4.0	76.0	3.1
64	-6.5 (n.s.)	4.1	76.8	3.1
LR_{50}	**> 64 kg a.s./ha**			
Reference item Dimethoate EC 400 30 mL product/ha (12 g a.s./ha)	61.3*	n.d.	n.d.	n.d.

1) Application rate in 200 L water/ha
2) Mortality after exposure to residues on treated bean leaves. The results for mortality in individual treatments were compared to that in the control using FISHER'S Exact Binomial test ($\alpha = 0.05$).
3) Corrected mortality according to ABBOTT (1925)

(n.s.) not statistically significantly different compared to the control
* statistically significantly different compared to the control
n.d. not determined

The results of the control group indicated that the test organisms were in a good condition (mortality: 22.5%, reproduction: 3.1 fertile eggs per viable female per day).
The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 61.3%).

Conclusions:
A calculation of the LR_{50} (median lethal rate) was not possible, since the corrected mortality of the test item groups did not exceed 6.5%. Therefore, the LR_{50} is empirically estimated to exceed the highest tested application rate, 64 kg a.s./ha.

The reproductive output was above the lower limit given as validity criterion (average number of fertile eggs per viable female per day in the control group > 2) according to the historical database of the ring testing group ([redacted] *et al.,* 2000). According to that, this parameter was considered as not impacted by the treatment.

Report:	KCP 10.3.2.2/04[redacted]; 2011; M-413058-01-1
Title:	Chronic toxicity (ER50) of Fosetyl-AL WG 80% w/w to the rove beetle Aleochara bilineata GYLL. under extended laboratory conditions
Report No.:	11 10 48 021 A
Document No.:	M-413058-01-1
Guideline(s):	IOBC Guideline (GRIMM et al. 2000) US EPA OCSSP 850.SUPP
Guideline deviation(s):	The relative humidity in the test room decreased, due to a technical fault, during the mortality phase on day 12/13 for 11 hours to 47% and during the reproduction phase on day 53/54 after application for 21 hours to 54% (the required range is 60-90%). These slight deviations did not affect the results of the study.
GLP/GEP:	**yes**

Materials and Methods:

The effects of the test item Fosetyl-Al WG 80% w/w [analysed active ingredient: 77.7% w/w Fosetyl-aluminium (LS 74783); Specification No.: 102000001579-03; Batch No.: LV38000066; Sample description: FAR01374-01, Material No.: 05921589] were tested under extended laboratory conditions after contact exposure of adults of the rove beetle *Aleochara bilineata* Gyll. to dried spray residues of the test item with rates of 28, 44 and 52 kg a.s./ha (equivalent to 36, 57 and 67 kg product/ha) in 400 L deionised water/ha applied onto sandy soil (LUFA 2.1). The control was treated with deionised water (400 L/ha). Dimethoate EC 400 (1.5 L product/ha, nominally equivalent to 600 g a.s./ha, in 400 L deionised water/ha) was used as a toxic reference item.

Adults of *Aleochara bilineata* Gyll. were exposed in 4 replicates per treatment group and 20 beetles per replicate to the spray residues of the test item, reference item and control treatments, respectively. During the assessments, the beetles were fed with deep frozen larvae of *Chironomus* spp.
The number of hatched beetles of the F_1 generation was recorded over a period of 68 days. From these data the endpoint reproductive capacity was calculated.

All validity criteria according to Grimm *et al.* (2000) for conducting the extended laboratory test with *Aleochara bilineata* were met.

Findings:

Test item	**Fosetyl-Al WG 80% w/w**				
Test organism	*Aleochara bilineata* GYLL.				
Exposure	Dried spray deposits on sandy soil (LUFA 2.1)				
Treatment	Reproductive capacity				
	Mean number of hatched beetles of the F_1-generation per replicate	Mean number of hatched beetles/ host pupa	Parasitisation rate (%)	Total number of hatched beetles of the F_1-generation per treatment group	Effect on reproductive capacity (relative to control)[2] R (%)
Control	573	0.382	38.2	2293	-
Application rate[1] [kg a.s./ha]					
28	531 (n.s.)	0.354	35.4	2124	7.4
44	477*	0.318	31.8	1907	16.8
52	448*	0.299	29.9	1791	21.9
Reference item Dimethoate EC 400 1.5 L product/ha (600 g a.s./ha)	10*	0.007	0.7	40	98.3

[1] Application rate in 400 L water/ha
[2] Effect on reproduction according to the following formula: (1-Pt/Pc) * 100% calculated on the exact raw data (positive values represent a decreased reproduction compared to the control)
n.s. = not statistically significantly different compared to the control: DUNNETT`s multiple t-test, $\alpha = 0.05$
* statistically significantly different compared to the control: DUNNETT`s multiple t-test (test item) or Student t-test (reference item), $\alpha = 0.05$

The results of the control group indicated that the test organisms were in a good condition (average number of hatched beetles of the F_1-generation per replicate: 573).
The results of the reference item group indicated that the test system was sensitive to harmful substances (98.3% reduction of reproductive capacity).

Conclusions:
In this extended laboratory test the effects of Fosetyl-Al WG 80% w/w residues on the reproductive capacity of the rove beetle *Aleochara bilineata* were determined at the rates of 28, 44 and 52 kg a.s./ha. The reduction of reproductive capacity at all tested rates was below 22%. The ER_{50} is >52 kg a.s./ha (equivalent to >67 kg product/ha).

CP 10.3.2.3 Semi-field studies with non-target arthropods

In view of the results presented in Sections CP 10.3.2.1 and CP 10.3.2.2, no semi-field studies were deemed necessary.

CP 10.3.2.4 Field studies with non-target arthropods

Report:	KCP 10.3.2.4/01 [redacted]s; 2010; M-367548-01-1
Title:	A field study to evaluate the effects of fosetyl-AL WG 80 on predatory mites (Acari: Phytoseiidae) in apple orchards in Southern France
Report No.:	S09-01000
Document No.:	M-367548-01-1
Guideline(s):	IOBC, BART and EPPO Guidance Document (BLUEMEL et al. 2000) BBA-Guideline Part VI, 23-2.3.4 (HEIMANN-DETTLEFSEN 1991) US EPA OCSPP 850.SUPP
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
Two field trials were carried out in an apple orchard to determine the effects of the fungicide Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) on the population development of predatory mites (*Acari: Phytoseiidae*).

Materials and Methods:
Test item: Fosetyl-Al WG 80 (818 g/kg analysed), specification No. 102000001579, batch ID EV38000066.

S09-01000-01
The trial S09-01000-01 included three treatment groups, one test item treatment with Fosetyl-Al WG 80, a water treated control and a reference item treatment.
The test item Fosetyl-Al WG 80 and the control were applied three times with a 7 days spay interval in mid-April. The target application rate for the test item was 3.6 kg/ha in 800 L/ha water for all applications. The reference item (Bulldock) was applied once at the 1st application of the test item at an application rate of 0.7 L/ha. The same amount of tap water used for the test item and reference item was applied in the control plots.

S09-01000-02
The trial S09-01000-02 included four treatment groups, two test item treatments with Fosetyl-Al WG 80, a water treated control and a reference item treatment.
The test item Fosetyl-Al WG 80 and the control were applied three times between mid-April and mid-May with a spray interval of 18 – 21 days. The target application rate was 4.0 kg product/ha for the first test item treatment (T1) and 7.2 kg product/ha for the second test item treatment (T2). The spray volume was adapted to the actual growth stage, starting with 800 L/ha for the 1st application up to 1200 L/ha at the 3rd application. The reference item (Bulldock) was applied two times, at the 1st and the 3rd application of the test item at application rates of 0.7 L/ha. The same amount of tap water used for the test item and reference item was applied in the control plots.

The population development of naturally occurring predatory mites was assessed in all treatment groups by determining the number of mites on leaf samples, using the washing method (Boller, 1984).

Results and Discussion:

S09-01000-01

In the pre-sampling the population density was between 0.45 and 0.50 predatory mites per leaf.
No relevant reduction of predatory mites was observed in the test item group (Fosetyl-Al WG 80) during the whole trial. The calculated effects of the test item on the predatory mite populations ranged between -25.0% and 1.7%.
For the reference item a maximum effect of 63.5% was observed. The mean number of predatory mites in the toxic reference item treated plots was statistically significant reduced when compared to the control in all post-application assessments (one sided Dunnett's t-Test, Wilcoxon Two-sample Test, $\alpha = 0.05$).

Summary of effects of Fosetyl-Al WG 80 and the reference item on predatory mites according to Abbott (1925):

Trial S09-01000-01		
Assessment before / after application no.	**Fosetyl-Al WG 80 [%] [5]**	**Reference Item [%] [4]**
4 DBA1	1.7	-8.9[2]
5 DAA3	-25.0[3]	63.5*[3]
26 DAA3	0.6[1]	44.9*[1]

DBA = Days before application, DAA = Days after application
* Statistically significant difference compared to the control
[1] Dunnett's t-Test ($\alpha = 0.05$), one-sided, with original data
[2] Dunnett's t-Test ($\alpha = 0.05$), one-sided, with transformed data
[3] Wilcoxon Two-Sample Test ($\alpha = 0.05$)
[4] Bulldock
[5] All effect values are generated with non-rounded mean values

S09-01000-02

In the pre-sampling the population density was between 0.49 and 0.53 predatory mites per leaf.
The effects of the test item Fosetyl-Al WG 80 applied three times at a rate of 4.0 kg/ha on the predatory mite populations ranged between 25.0% and -32.4%. A maximum effect of 25.0% was observed in the 4th assessment (5 DAA3). In the last assessment (26 DAA3) an effect of 7.6% was observed.
The effects of the test item Fosetyl-Al WG 80 applied three times at a rate of 7.2 kg/ha on the predatory mite populations ranged between 39.9% and -42.5%. A maximum effect of 39.9% was observed in the 3rd assessment (9 DAA2). In the last assessment (27 DAA3) an effect of 25.2% was observed.
For the reference item a maximum effect of 86.6% was achieved. The mean number of predatory mites in the toxic reference item treated plots was statistically significant reduced when compared to the control in all post-application assessments (Dunnett's t-Test, one-sided, $\alpha = 0.05$).

Summary of effects of Fosetyl-Al WG 80 and the reference item on predatory mites according to Abbott (1925):

Trial S09-01000-02			
Assessment before / after application no.	**Fosetyl-Al WG 80 3 x 4.0 kg/ha [%]**[2)]	**Fosetyl-Al WG 80 3 x 7.2 kg/ha [%]**[2)]	**Reference Item [%]** [1) 2)]
4 DBA1	3.7	-3.1	-1.6
9 DAA1	-32.4	-42.5	77.2*
9 DAA2	-6.0	39.9*	77.4*
5 DAA3	25.0*	29.0*	86.6*
26 DAA3	7.6	25.2*	64.2*

DBA = Days before application, DAA = Days after application
* Statistically significant difference compared to the control (Dunnett's T-test, one-sided, $\alpha = 0.05$)
[1)] Bulldock
[2)] All effect values are generated with non-rounded mean values

Conclusions:
Based on the results of this study and according to the corresponding guidelines (Heimann-Detlefsen, 1991; [redacted] *et al.*, 2000), no unacceptable effects on predatory mite populations (Acari: Phytoseiidae) were observed if Fosetyl-Al WG 80 was applied three times at an application rate of 3.6 kg/ha and a spray interval of 2 days, or applied three times at an application rate of 4.0 kg/ha and a spray interval of 18 - 21 days as well as at an application rate of 7.2 kg/ha and a spray interval of 18 - 21 days in an apple orchard in Southern France.

Request from the RMS:
In the study from [redacted], J.; 2010; M-367548-01-1), 70-75 flower bud clusters before the applications and 150 leaves for the first sampling post-application had to be sampled to reach or to be closed to the recommended density of 30 mites. This indicates a low density of the mite populations since according to the guideline [redacted] et al. (2000) the density of 30 mites could be reached with sample of 25 leaves. Could you please provide further data to support the robustness and the reliability of the results from this study?
In addition, it is RMS opinion that some significant effects without recovery at the end of the test are observed for 3 applications at a rate of 7.2 kg product/ha.

Response from BCS:
The guideline requests that a "minimum" of 25 leaves should be sampled per replicate. Furthermore, if the mean number of mites per replicate in the untreated control is less than 30 the number of sampled leaves should be increased. This has been done in the study in line with the guideline requirements.
The number of mites per replicate in trail S09-01000-**01** is provided in Table 41 of the report. Calculating the mean number of mites for the control treatment results in values of 34.6, 44.8, and 129.4 mites/sample for the 3 sampling dates. This indicates that the sample size was in line with the guideline requirements. The statistical evaluation is based on the number of mites per replicate and the number of leave does not influence the evaluation. The results are therefore considered as reliable.
The number of mites per replicate in trail S09-01000-**02** is reported in Table 42 of the report. The mean numbers of mites for the control treatment are 38.4, 27.8, 173.8, 136.8, and 158.8 mites/sample. The pre-application sampling (38.4 mites/sample) and the 2nd, 3rd, and 4th post-application samplings (173.8, 136.8, and 158.8 mites/sample, respectively) clearly meet the guideline requirement. Only for the first post-application sampling the mean number of mites was slightly below (-7%) the recommended minimum number. Since the pre-application density and the 2nd to 4th post-application density assessments in the control clearly meet the guideline requirements that study can be considered as reliable since the statistical evaluation is based on the number of mites per replicate and the number of leave does not influence the evaluation.

At the end of in trail S09-01000-**02** there was still a statistically significant difference between the control and the 3 x 7.2 kg/ha treatment group. Even though statistically significant the difference was only 25.2%. According to the IOBC/WPRS criteria are effects in field studies classified as harmless or slightly harmful if the observed effects are in the range of 0 to 50%. In line with this classification requires the test guideline for predatory mites ([redacted] *et al.*, 2000) that the sampling period needs only to be prolonged if the calculated treatment effect differs by more than 50% from the control. It can be concluded that a difference of 25.2% observed in a predatory mite field study is not biological relevant.

Report:	KCP 10.3.2.4/02[redacted]カ; 2013; M-475378-01-1
Title:	A field study to evaluate the effects of fosetyl-Al WG 80 percent w/w on predatory mites (Acari: Phytoseiidae) in an apple orchard in Germany
Report No.:	S13-01518
Document No.:	M-475378-01-1
Guideline(s):	IOBC, BART and EPPO Guidance Document (BLÜML et al., 2000)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Objective:
The purpose of this study was to investigate short and long term effects of the test item Fosetyl-Al WG 80% w/w on the population development of leaf-dwelling phytoseiid mites (Acari: Phytoseiidae) in an apple orchard.

Material and Methods:
Fosetyl-Al WG 80% w/w (nominal content of active ingredient): Fosetyl-Aluminium 81.0% (analysed content of active ingredient); Specification No. ID20000024225-01; Batch No.: EV36003202
The trial included six treatment groups, three test item treatment groups (T1, T2 and T3), two water treated control groups (C1 and C2) and a reference item treatment group R. The test items were applied at three different rates and time intervals between beginning and mid of June 2013.
Test item treatment T1: 3 x 4.5 kg product/ha with an application interval of 3-4 days
Test item treatment T2: 3 x 7.5 kg product/ha, with an application interval of 7 days
Test item treatment T3: 3 x 3.75 kg product/ha, with an application interval of 9 days
All spray applications were conducted with an application volume of 1000 L water/ha. The control treatment C1 was applied at each application of T1 and T2, the control treatment C2 was applied at each application of T3. The reference item, Karate Zeon, (0.075 L/ha) was applied two times between beginning and mid of June 2013, at the first and the last application of the test item treatment T2. The field site was located in [redacted], Germany.
Natural occurring populations of predatory mites (Acari: Phytoseiidae) were exposed to three applications of the test item in an apple orchard at three different rates. The population development of predatory mites was assessed by determining the total number of mites on leaf samples.

Dates of experimental work: May 21, 2013 – September 19, 2013

Results:
The mean number of mites per leaf after the first application was between 0.38 and 0.57 in the control treatment C1, between 0.33 and 0.73 in the control treatment C2, between 0.31 and 0.40 in the test item treatment T1, between 0.30 and 0.40 in the test item treatment T2, between 0.32 and 0.52 in the test item treatment T3 and between 0.00 and 0.03 in the reference item treatment R.
The effect of the test item on the predatory mite populations ranged between 12.1% and 26.0% for test item treatment T1, between 12.4% and 42.3% for test item treatment T2 and between -40.2% and 29.0% for test item treatment T3. The difference between the predatory mite numbers observed in the plots treated with the test item was not statistically significant compared to the control at any sampling date. The maximum effect was 26.0% for test item treatment T1 at the third sampling date S3

(6 DAA3/4), 42.3% at the last sampling for test item treatment T2 (30 DAA6) and 29.0% at the last sampling (26 DAA7) for test item treatment T3.
In the plots treated with the reference item a maximum reduction of 100.0% was observed at seventh sampling date S7 (27 DAA3/4). The reduction in the reference item treatment was statistically significant at all assessments after the first application (Pooled T-test, Satterthwaite T-test or Wilcoxon test, all two sided with α = 0.05).

Summary of effects of test item and the reference item on predatory mites according to Abbott (1925)

Sample Code	Timing / Date	Test item group T1 [%] [4]	Test item group T2 [%] [4]	Test item group T3 [%] [4]	Reference item R [%] [4]
S1	14 DBA1 21/05/2013	-1.9	-0.3	-1.6	-0.1
S1-R	0 DBA1 04/06/2013	23.6	16.1	-2.4	6.2
S2	3 DAA2 / 6 DAA1 10/06/2013	12.1	12.4	-40.2	94.1*[1]
S3	6 DAA3/4 17/06/2013	26.0	23.5	-	97.9*[2]
S4	7 DAA5 20/06/2013	-	-	5.8	97.8*[2]
S5/6[5]	9 DAA6 / 5 DAA7 27/06/2013	-	19.9	20.3	97.6*[2]
S7	27 DAA3/4 08/07/2013	21.4	-	-	100.0*[2]
S8/9[5]	30 DAA6 / 26 DAA7 18/07/2013	-	42.3	29.0	98.4*[3]

DBA = Days before application, DAA = Days after application, S1-R: Repetition of S1
* Statistically significant difference compared to the control
[1] Pooled T-test, two sided (α = 0.05)
[2] Satterthwaite T-test, two sided (α = 0.05)
[3] Wilcoxon test, two sided (α = 0.05)
[4] All effect values are generated with non-rounded mean values
[5] Samplings S5 and S6 and sampling S8 and S9 were performed on the same day

Conclusion:
Based on the results of this study and according to the corresponding guideline ([redacted] *et al.*, 2000), no unacceptable effects on predatory mite populations (Acari: Phytoseiidae) were observed when the test item Fosetyl-Al WG 80% w/w was applied 3 times at application rates of 4.5 kg/ha, 7.5 kg/ha and 3.75 kg/ha in 1000 L water/ha in an apple orchard.

Request from the RMS:
In the study from [redacted] ([redacted], D., 2013, M-475378-01-1), the number of mites observed per sample (150-200 leaves per sample) seems to indicate a low density of mites. Could you please provide further data to support the robustness and the reliability of the results from this study?
In addition the observed effects up to 26% (T1: 3 x 4.5 kg product/ha), 42.3% (T2: 3 x 7.5 kg product/ha) and 29% (T3: 3 x 7.5 kg product/ha) are not significantly different from the control. Further explanations are required to support that these effects would not have to be considered as biologically significant.

Response from BCS:
The guideline requests that a "minimum" of 25 leaves should be sampled per replicate. Furthermore, if the mean number of mites pre replicate in the untreated control is less than 30 the number of sampled leaves should be increased. This has been done in the study in line with the guideline requirements.
Table 32 of the report is listing the number of mites per sample. All single control replicates exceeded the number of 30 mites/sample. All mean numbers per sample are between 50 and 110 mites and do clearly exceeding the guideline requirement of a mean value of 30 mites per sample. The statistical evaluation is based on the number of mites per replicate and the number of leave does not influence the evaluation. The results are therefore considered as reliable.
Concerning the observed differences of T1, T2, T3 in the range of 26 to 42.3% in needs to be considered that the test design is appropriate to detect effects of more than 50% as statistically significant in 90% of the cases as stated in the guideline ([redacted] *et al.*, 2000). Furthermore, does the predatory mite field study guideline indicates that the assessment needs only to be prolonged if the effects observed in the treatment group exceed 50%.
According to the IOBC/WPRS criteria are effects in field studies classified as harmless or slightly harmful if the observed effects are in the range of 0 to 50%. The observed differences are therefore not considered as biological relevant.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No relevant exposure of non-target arthropods is expected by other routes of exposure.

CP 10.4 Effects on non-target soil meso- and macrofauna

The risk assessment procedure follows the requirements as given in the Council Directive 91/414/EEC (Annex III), Council Directive 97/57/EC (Annex VI) and the Guidance Document on Terrestrial Ecotoxicology.

Predicted environmental concentrations used in risk assessment
The PEC_{soil} values below are taken from Document MCP, Section 9.1.3.

Table 10.4-1: Initial max PEC_{soil} values (bold values were used in the tier 1 risk assessment)

Compound	Orchards
	$PEC_{soil, max}$
Fosetyl-Al	**1.920 mg a.s./kg dws**
Phosphonic acid	3.930 mg pm/kg dws

Table 10.4-2: $PEC_{soil\ accu}$ values (mixing depth of 5 cm for plateau calculation; bold values were used in the tier 1 risk assessment)

Compound	Orchards	
	$PEC_{soil, plateau}$ [mg/kg]	$PEC_{soil, accu}$ [a] [mg/kg]
Phosphonic acid	2.532 mg pm/kg dws	**6.462 mg pm/kg dws**

[a] $PEC_{soil, accu}$ means the sum of $PEC_{soil, max}$ and $PEC_{soil, plateau}$

CP 10.4.1 Earthworms

Table 10.4.1- 1: Endpoints used in risk assessment

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 **234.4 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], U., 1999; M-189218-01-1 KCA 8.4.1/01
	Eisenia fetida Reproduction, 56 d, mixed	NOEC	693 mg pm/kg dws	[redacted], T.; 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%.

Bold values: endpoints used for risk assessment

Table 10.4.1- 2: Ecotoxicological endpoints – earthworm field study with formulated product

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Fosetyl-Al WG 80	Natural earthworm fauna, field study 1 year, long-term plateau was included in 1st application, 2nd and 3rd application at 29 and 30 days after 1st application	**NOEC**	**184.1 kg prod./ha (pc + 1st application of ar) + 2 x 97.8 kg prod./ha (2nd and 3rd application of ar)**	[redacted],; 2010; M-398002-01-1 KCP 10.4.1.2/01

Risk assessment for earthworms

Table 10.4.1- 3: TER calculations for earthworms

Compound	Species, study type	Endpoint [mg/kg]		worst case $PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Fosetyl-Al WG 80	Earthworm, reproduction	NOEC	254.4	1.920	132.5	5
Phosphonic acid	Earthworm, reproduction	NOEC	≥ 498.79	6.462	≥ 77.2	5

All TER values calculated with the worst case $PEC_{soil, max}$ values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on earthworms are to be expected from the intended uses of Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80). This conclusion is also in line with the results of an available earthworm field study ([redacted], G.; 2010; M-398002-01-1) which indicated no unacceptable effects after the application of Fosetyl-Al WG 80 at a rate of 184.1 kg prod./ha followed by 2 applications of 97.8 kg prod./ha.

CP 10.4.1.1 Earthworms sub-lethal effects

Please refer to Document MCA, Section 8.4.1.

CP 10.4.1.2 Earthworms field studies

Report:	KCP 10.4.1.2/01 [redacted],; 2010; M-398002-01-1
Title:	Fosetyl-aluminium WG 80C W: Effect of spray application on the earthworm Fauna within one year
Report No.:	R09-152
Document No.:	M-398002-01-1
Guideline(s):	ISO 11268-3 (1999): Soil quality Effects of pollutants on earthworms Part 3: Guidance on the determination of effects in field situations; [redacted] (2006): Technical recommendations for the update of the ISO Earthworm Field Test Guideline (ISO 11268-3); ISO 23611-1 (2007): Soil quality Sampling of soil invertebrates - Part 1: Hand-sorting and formalin extraction of earthworms
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of the current study was to identify possible effects of the fungicide Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) on a natural site-specific earthworm community on a grassland area in Germany.

Materials and Methods:
Test item: Fosetyl-Al WG 80 (818 g/kg fosetyl-aluminium analysed), batch ID EV38000066.

The study was conducted between 27.04.2009 and 27.04.2010 on grassland (meadow) near [redacted] ([redacted]), Germany. The study design comprised the test item with three applications (first application 184.1 kg (plateau concentration[2] + single application rate), second and third application 97.8 kg Fosetyl-Al WG 80/ha), a reference item (approx. 10.0 kg carbendazim/ha) and a control (tap water). For the reference item only one application (0 DAA) was performed. The control was treated with tap water at each of the three applications. Each treatment consisted of four replicates. Earthworms were sampled using a combination of excavation/hand-sorting and formalin extraction. Samplings were conducted once before application (pre-sampling, 10 DBA) and three times after the first application (approx. one month (39 DAA), five months (148 DAA) and approx. one year (333 DAA)). Earthworm data were analysed using for the one test item rate recommended non-parametric (Mann-Whitney-U-Test) statistical procedures.

The pre-sampling on the selected site showed that the criteria recommended by [redacted] *et al*. (2006) for conducting earthworm field studies were met:
1. Total density ≥ 100 earthworm individuals/m²;
2. Sufficient abundance of representatives of the two ecological earthworm groups 'anecics' (*Lumbricus terrestris*) and endogeics' (*Allolobophora chlorotica, Aporrectodea caliginosa*). Further, homogeneity of the study field was confirmed at the pre-sampling, *i.e.* no significant differences between any of the three treatment groups occurred.

[2] Estimated concentration level after repeated use of the test item

After the first application, the measured residues of the phosphonic acid in the soil samples were within the recommended range of 50 to 150% (67.6%). The results of the soil samples taken after 2nd and 3rd application needed to be corrected by the residue concentration existing in the soil prior to the respective application. This resulted in recovery rates of 79 and 32.1% of the nominal desired application rate (converted to phosphonic acid equivalents). The low recovery rate after the last application could be explained by the high variability of the replicates (-22, 68, 22 and 61% corrected recovery) and other factors that possibly influenced this recovery rate. However the analysis of the spray solutions from each application confirmed that the actual desired test item concentration was correctly applied to the study field (± 10% of nominal concentration). Hence exposure of the earthworms to the desired test item concentrations could be confirmed for all three applications.

Findings:
Earthworm abundance of the study field was sufficient at each sampling event (230, 200, 131 and 224 individuals/m^2 in control plots at pre-sampling, first, second and third sampling, respectively, see Table below). The earthworm community comprised eight different species, representing all ecological groups (epigeic, endogeic and anecic earthworms). Three of these species (*Allolobophora chlorotica*, *Aporrectodea caliginosa* and *Lumbricus terrestris*) were analysed separately because their abundance exceeded 10 individuals/m² in the control plots at least during one sampling event after application.
No dead or moribund earthworms were found on the soil surface of test item treated study plots after each application. The total biomass of earthworms was significantly reduced in the reference item treatment by 51, 64 and 34% compared to the control at the first, second and third post-application sampling, respectively. Further, the total number of earthworms was significantly reduced by 23% at the third post-application sampling.
In the test item treatments no statistically significant adverse effects on abundance or biomass of total earthworm population, total adult and total juvenile earthworms, morphological groups (tanylobous and epilobous) or single species (representatives of anecic and endogeic earthworms), could be detected at any of the three post-application sampling events (approx. one month, five months and approx. one year).

Summary Table: Mean abundance and biomass of the total earthworm population, total juveniles, total adults and of the dominant species, *L. terrestris, A. chlorotica* and *A. caliginosa* in the control, the test item treatments and the reference item treatment

Mean (n=4); In brackets: the percentages compared to control; Statistic: comparisons test item treatments *vs.* control: Mann-Whitney-U-Test; comparisons reference *vs.* control: Mann-Whitney-U-Test; bold: significant from control ($p \leq 0.05$)

		Abundance (Individuals/m²)				Biomass (g/m²)			
		Sampling				Sampling			
	Treatment	Pre[a]	1[b]	2[c]	3[d]	Pre[a]	1[b]	2[c]	3[d]
Total earthworms	Control	230.3	199.8	130.5	224.3	107.8	94.3	57.7	140.5
	Fosetyl-Al	242.5 (105.3%)	196.0 (98.1%)	163.5 (125.3%)	245.5 (109.5%)	112.8 (104.7%)	88.7 (94.0%)	65.9 (114.4%)	121.4 (86.4%)
	Reference	249.3 (108.3%)	152.0 (76.1%)	70.3 (53.8%)	**172.3 (76.8%)**	113.8 (105.6%)	**46.4 (49.2%)**	**21.0 (36.5%)**	**93.5 (66.5%)**
Total adults	Control	91.8	67.8	61.3	85.5	62.1	52.1	42.6	88.0
	Fosetyl-Al	113.0 (123.2%)	73.3 (108.1%)	72.3 (118.0%)	91.5 (107.0%)	72.0 (116.0%)	51.9 (99.7%)	45.7 (107.2%)	75.7 (86.1%)
	Reference	106.3 (115.8%)	48.8 (72.0%)	30.0 (49.0%)	92.3 (107.9%)	68.7 (110.7%)	**18.1 (34.9%)**	**13.1 (30.7%)**	62.6 (71.1%)
Total juvenil	Control	124.5	126.5	65.3	132.8	41.1	39.7	13.6	46.2
	Fosetyl-Al	117.5 (94.4%)	116.8 (92.3%)	83.8 (128.4%)	146.8 (110.5%)	36.8 (89.6%)	33.6 (84.6%)	17.6 (128.9%)	39.4 (85.2%)
	Reference	134.8 (108.2%)	94.5 (74.7%)	37.0 (56.7%)	**74.3 (55.9%)**	41.4 (100.9%)	**24.2 (61.0%)**	6.6 (48.6%)	**23.1 (50.0%)**
Allolobophora chlorotica **(endogeic)**	Control	66.5	55.0	17.5	46.3	7.9	6.5	1.8	8.5
	Fosetyl-Al	64.0 (96.2%)	56.0 (101.8%)	24.8 (141.4%)	81.3 (175.7%)	8.1 (103.0%)	6.9 (106.7%)	2.7 (155.6%)	13.7 (161.6%)
	Reference	68.3 (102.6%)	47.0 (85.5%)	10.5 (60.0%)	37.5 (81.1%)	8.3 (105.8%)	6.6 (102.0%)	1.1 (62.9%)	7.7 (90.3%)
Aporrectodea caliginosa **(endogeic)**	Control	18.0	17.8	36.0	36.5	10.0	10.8	13.9	32.0
	Fosetyl-Al	26.0 (144.4%)	20.0 (112.7%)	42.0 (116.7%)	30.5 (83.6%)	14.9 (148.9%)	12.3 (113.9%)	17.7 (127.2%)	25.0 (78.2%)
	Reference	21.0 (116.7%)	13.0 (73.2%)	21.0 (58.3%)	54.8 (150.0%)	12.2 (121.6%)	8.0 (73.7%)	8.8 (63.1%)	49.1 (153.4%)
Lumbricus terrestris **(anecic)**	Control	23.5	22.0	16.0	23.3	56.3	44.6	29.8	55.9
	Fosetyl-Al	26.8 (113.8%)	23.5 (106.8%)	15.5 (96.9%)	18.0 (77.4%)	56.9 (101.0%)	44.5 (99.8%)	28.0 (94.1%)	45.6 (81.5%)
	Reference	27.0 (114.9%)	**4.5 (20.5%)**	**2.0 (12.5%)**	**3.0 (12.9%)**	56.5 (100.3%)	**6.8 (15.2%)**	**3.2 (10.8%)**	**4.2 (7.5%)**

a= Pre-treatment sampling (10 days before application)
b= 1st post-treatment sampling (~1 month after application)
c= 2nd post-treatment sampling (~5 months after application)
d= 3rd post-treatment sampling (~12 months after application)

Conclusions:

The current study meets all criteria required for a valid earthworm field study as requested by the available guidance for earthworm field studies (ISO 11268-3 1999; [redacted] *et al*., 2006). At three sampling dates (39, 148 and 333 DAA) after three applications of the test item Fosetyl-Al WG 80 (a.s. fosetyl-aluminium) the abundance and biomass of earthworms were not significantly affected. Therefore, it can be concluded that application of 1x184.1 kg (plateau concentration + single application rate) and two following applications of 97.8 kg Fosetyl-Al WG 80/ha have no adverse effects on natural earthworm field communities.

Request from the RMS:

Further results of the study from [redacted] ([redacted], G.; 2010; M-398002-01-1) should be further justified. Without these precisions the reliability of this study could be challenged during the peer-review process.

a) The recoveries of the soil concentrations after the third application are quite low and below the trigger of 50%. One replicate indicates a negative recovery (-22%) which is difficult to understand without further explanations.
b) More details on the statistical analysis of the results would be suitable since some important effects of the reference are not statistically significant (i.e. 51% of effects on total adult abundance at sampling 2).
c) The relevance of the lower abundance (83.6%) and biomass (78.2%) of *Aporrectodea caliginosa* compared to the control at the last sampling date should be discussed.
d) The trend of effects on Lumbricus terrestris should be discussed as the abundance and the biomass of this species had a decreasing percentage compared to the control decrease during the study duration.

Response from BCS:

a) The amount of test substance fosetyl-Al actually applied is given in Table 10 of the study report. The rates can be derived from volumes of spray solutions, the content of active substance in spray solution as analytically verified, and the residual volumes remaining in the spray tanks after application. The data allow for the calculation of the total rate of fosetyl-Al applied to the field. The actual application rates (mean amounts of test item per hectare) of the test item Fosetyl-Al WG 80 at the three applications were thus well within the range of ±10% of target amount for each application (see Table 10) indicating that the amounts actually applied to the field were correct.

With fosetyl-Al undergoing very fast microbial transformation in soil, phosphonic acid was chosen as analytical target to describe the exposure of earthworms to the relevant residues from use of fosetyl-Al.

However, phosphonic acid proved to be a difficult analytical target undergoing spontaneous fixation to soil particles following its contact with the soil matrix. Being called as ‘ageing’ of residues it is a common observation for ‘organic’, i.e. carbon containing chemicals, but also observed for ‘inorganic’ compounds like phosphonates and phosphates. The phenomenon was also observed in aerobic soil degradation tests.

The analytical method for determination of phosphonic acid was fully validated according to actual standards for two methods of extraction, i.e. use of aqueous ammonium or aqueous sulfuric acid as solvents. In trend, recoveries were higher for lower test concentrations in soil and from use of sulfuric acid to be in the acceptable range of 69 to about 100%.

Sufficient rainfall (the amount was set to the standard minimum rainfall amount of 10 mm/m^2) was regarded as necessary to ensure the transport of fosetyl-Al residues from the grass to soil and to enable the full formation of phosphonic acid from fosetyl-Al.

Residues of fosetyl-Al were allowed in this study to age typically for 2 to 3 days after application in the field before soil sampling. However, since phosphonic acid was not applied directly to the field, the formation process (separation from formulation ingredients, change of physico-chemical characteristics from fosetyl-AL to phosphonic acid) increased the variability (i.e. inhomogeneity) of the distribution of the analyte in soil samples under conditions of the field. Moreover, the ageing

period to fulfill the 10 mm criterion reduced the recovery of phosphonic acid significantly by, as indicated above, fast formation of non-extractable residues in the silty clay field soil.
While the use of ammonia as a mild solvent did not result in high recoveries from this silty clay field soil, extraction has to be regarded as harsh by use of sulfuric acid (0.5 M). Its use enhanced recoveries, but the harsh extraction resulted in darker colored extracts difficult to analyze. The use of an even harsher extraction method is not recommended since it would further degrade or disturb the soil matrix which is clearly not the objective in development of analytical methods.
A calculation of residues after each application and shortly before the next application was presented in Table 7 within section 5.3 of the analytical phase report in the attachment at the end of the report.
In this study, the recovery values of the 2nd and the 3rd application were calculated each by subtraction of the phosphonic acid concentration value measured directly before application from the value obtained after application ('differencial calculation'). They were given in Table 13 of the report. For illustration in this document a table is composed from the two tables mentioned above in which the calculated recovery values from Table 13 are added on the right to Table 7 from section 5.3 (see below).
Following the 1st application, the recovery of phosphonic acid was 67.6% of the nominal value (average of all replicates, value corrected by analytical recovery of 91%). Following the second application, the recovery was 79% (average of all replicates, value corrected by analytical recovery of 91%). These two values are thus well above the threshold of 50%.
Unfortunately, the differential calculation for values of phosphonic acid recovered after the third application in comparison to the recovery 3 days before the last treatment resulted in case of replicate T1 in a negative calculated value (-22% recovery) and in case of T3 in a lower value than expected (+22% recovery). The values of the other two replicates (+68% and +61% recovery) were in the range of recoveries expected from the 1st and the 2nd application.
The inconsistencies in these phosphonic acid recovery values for the 3rd application can be well explained by a certain inhomogeneity during formation and most importantly by the rapid fixation of phosphonic acid residues to the field soil during ageing thereby reducing the extraction efficiency.

The table shown below for illustration is created from Table 7 in section 5.3 of the analytical phase report included in the study. In the table the ammonia extract values and the control values (all below detection limit) were omitted. Additionally, the calculated phosphonic acid recovery values are included as columns on the right:

Table 7: Analytical results for fosetyl-Al and phosphonic acid in dry soil

Sample ID	Plot code	Sampling date	Comment	Fosetyl-Al	Phosphonic acid (sulfuric acid extract) Values per plot [mg/kg]	Phosphonic acid (sulfuric acid extract) Mean values [mg/kg]	Nominal calculated concentration value per application [mg/kg]	Recovery of phosphonic acid from soil after subtraction of value before application [%]
69	T1	11.05.2009	4 days after 1st application (2009-05-07)	< LOQ	43.2	41.4*	67.6	67.6% *
70	T2	11.05.2009		< LOQ	40.5			
71	T3	11.05.2009		< LOQ	42.7			
72	T4	11.05.2009		< LOQ	39.3			
63	T1	04.06.2009	1 day before 2nd application (2009-06-05)	< LOQ	30.5	31.9*		
73	T2	04.06.2009		< LOQ	33.7			
74	T3	04.06.2009		< LOQ	36.0			
75	T4	04.06.2009		< LOQ	27.5			

Sample ID	Plot code	Sampling date	Comment	Fosetyl-Al	Phosphonic acid (sulfuric acid extract) Values per plot [mg/kg]	Phosphonic acid (sulfuric acid extract) Mean values [mg/kg]	Nominal calculated concentration value per application [mg/kg]	Recovery of phosphonic acid from soil after subtraction of value before application [%]
85	T1	08.06.2009	3 days after 2[nd] application (2009-06-05)	< LOQ	56.3	59.3*	38.6	79.0% *
86	T2	08.06.2009		< LOQ	76.9			
87	T3	08.06.2009		< LOQ	46.9			
88	T4	08.06.2009		< LOQ	57.1			
144	T1	03.07.2009	3 days before 3[rd] application (2009-07-06)	< LOQ	55.9	47.5*		
145	T2	03.07.2009		< LOQ	40.9			
146	T3	03.07.2009		< LOQ	49.1			
147	T4	03.07.2009		< LOQ	44.2			
157	T1	08.07.2009	2 days after 3rd application (2009-07-06)	< LOQ	48.6	58.3*	37.2	-22% **
158	T2	08.07.2009		< LOQ	63.7			68% **
159	T3	08.07.2009		< LOQ	56.3			22% **
160	T4	08.07.2009		< LOQ	64.5			61% **

* Mean values
** Individual values for each replicate; Mean value for all replicates = 32.1%

Effects of the toxic reference on earthworms were statistically evaluated by the Mann Whitney U-test (separate from the analysis of the test item) as recommended by [redacted] *et al.* 2006. Effects were statistically not significant as the variability of the abundances in the reference treatment group is relatively high (CV = 71.8%). However, the CV in the control was relatively low with 36.7%. The observed variability of total adult abundances in the control are well in the range of the natural variability of earthworm populations in the field.
However, the strong statistically significant effects of the reference treatment on biomass of total earthworms and total adult earthworms at the 1[st] and 2[nd] sampling confirm the overall sensitivity and the validity of the test system as recommended by [redacted] *et al.* 2006.

The observed reduction of the abundance *A. caliginosa* by the test item compared to the control 1 year after application is clearly within the range of natural variability and cannot be regarded as an adverse effect. The abundances of *A. caliginosa* in the period of one year in the control shows a mean CV = 36.64. Reductions of 16.4 and 21.8% cannot be reliably interpreted as an adverse effect on earthworm populations. Furthermore, reductions in abundances of *A caliginosa* were neither seen at the 1[st] sampling nor at the 2[nd] sampling after application. Thus, these reductions are not considered being treatment related.

The development of the abundances of *L. terrestris* compared to the control during the year with 107, 97, and 77.4% compared to the control should not be interpreted as a trend (same for the biomass data). The observed reduction of 22.6% of the abundance of *L. terrestris* in an earthworm field study at the end of the study is not an adverse effect as this difference to the control is low (compared to the usual variability in the field) and statistically not significant. Differences observed in this order of magnitude represent the noise of the test system and cannot be reliably interpreted as an effect.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Table 10.4.2- 1: Endpoints used in risk assessment

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], S.; 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], S.; 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], L.; 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] M. I.; 2015; M-532897-01-1 KCA 8.4.2.1/04

dws = dry weight soil; a.s. = active substance; pm = pure metabolite; prod. = product
Bold values: endpoints used for risk assessment

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

Table 10.4.2- 2: TER calculations for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg/kg]		$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Fosetyl-Al WG 80	*Folsomia candida*	NOEC	452.4	1.920	235.6	5
	Hypoaspis aculeifer	NOEC	≥ 805		≥ 419.3	
Phosphonic acid	*Folsomia candida*	NOEC	≥ 1000	6.462	≥ 154.8	
	Hypoaspis aculeifer	NOEC	≥ 1000		≥ 154.8	

All TER values calculated with the worst case $PEC_{soil,\ max}$ values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80).

CP 10.4.2.1 Species level testing

Please refer to Document MCA, Section 8.4.2.1.

CP 10.4.2.2 Higher tier testing

In view of the results presented in Section CP 10.4.2, no further testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

Table 10.5- 1: Endpoints used in risk assessment

Test item	Test design	Endpoint		Reference
N-transformation				
Fosetyl-Al	Study duration 28 d	no unacceptable effects	20.0 kg a.s./ha **26.6 mg a.s./kg dws**	[redacted], J.; 1998; M-184321-01-1 KCA 8.5/01
Fosetyl-Al WG 80	Study duration 42 d	no unacceptable effects	978 kg prod./ha 1304 mg prod./kg dws **1067 mg a.s./kg dws**	[redacted], T.; 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], L.; 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
Bold values are used in the risk assessment

Risk assessment for Soil Nitrogen Transformation

Table 10.5- 2: Risk Assessment for soil micro-organisms

Compound	Species	Endpoint [mg a.s./kg]	$PEC_{soil,max}$ [mg a.s./kg]	Refinement required
Fosetyl-Al	Soil micro-organisms	26.60	1.920	No
Fosetyl-Al WG 80	Soil micro-organisms	1067	1.920	No
Phosphonic acid	Soil micro-organisms	65.31	6.462	No

According to regulatory requirements the risk is acceptable, if the effect on nitrogen transformation at the maximum PEC_{soil} values is < 25% after 100 days. In no case, deviations from the control exceeded 25% after 28 up to 42 days, indicating low risk to soil micro-organisms.

CP 10.6 Effects on terrestrial non-target higher plants

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Spray drift from the treated areas may produce residues of a product in adjacent off-crop areas.
Tier 1 limit tests have been conducted with the formulation Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) according to OECD testing guideline 208B (vegetative vigour study from 2000) and OECD testing guideline 208 (seedling emergence study from 2015). For the vegetative vigour study submitted for the Annex I inclusion of fosetyl under Directive 91/414/EEC, please refer to the Baseline Dossier provided by Bayer CropScience and the DAR. Additionally, a short summary is provided in grey typeface in Section CP 10.6.2 in this Supplementary Dossier. An additional Tier 1 study on seedling emergence 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. A summary of this study can be found in Section CP 10.6.2.
Since study results were originally reported on an active substance basis, the summary of endpoints and subsequent TER calculations are provided on an active substance basis as well.
The findings from these studies are summarised in the following table.

Table 10.6- 1: Endpoints used in risk assessment

Test organism	Study type	Max. effects	Most sensitive species	References
Maximum application rate: 80 kg a.s./ha				
Terrestrial non-target plants; 6 species	Vegetative vigour; Tier 1 single dose Tier 2 with cabbage and tomato 21 days	No effects ≥ 25 % at a rate of 80 kg a.s./ha (Tier 1) Tomato: EC_{25} = 8.6 kg a.s./ha; EC_{50} > 80 kg a.s./ha (Tier 2)	Tomato	[redacted].; 2000; M-199640-01-1 KCP 10.6.2/01
Maximum application rate: 7 kg a.s./ha				
Terrestrial non-target plants; 10 species	Seedling emergence; Tier 1 single dose 21 days	33.3% inhibition of emergence at a rate of 7 kg a.s./ha	Oilseed rape	[redacted].; 2015; M-525769-01-1 KCP 10.6.2/02

In the case of Fosetyl-Al WG 80, the tier 1 vegetative vigour study showed no phytotoxic effects >50% at the tested rate of 80 kg a.s./ha (equivalent to 100 kg product/ha). The tier 1 seedling emergence study showed no phytotoxic effects >50% at the tested rate of 7 kg a.s./ha.

Risk assessment for Terrestrial Non-Target Higher Plants

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. To demonstrate the low risk of the formulation to non-target plants, TER calculations have been performed for the representative use in orchards. The test rate of 7 kg a.s./ha was used as a most conservative endpoint estimate (ER_{50} > 7 kg a.s./ha). For three applications to pome fruit 11.01% of the full application rate of 3.6 kg a.s./ha are assumed to reach areas at 3 m from the edge of the crop. The amount of spray drift from three applications reaching off-crop habitats is calculated using the 77[th] percentile estimates derived by the BBA (2000)[3] from spray-drift predictions of Ganzelmeier & Rautmann (2000)[4]. According to Table 13 in the Guidance document for Risk Assessment on Birds and Mammals (EFSA, 2009[5]) a multiple application factor of 2.0 (MAF_{mean}) has to be applied for three applications in pome fruit with a 7 d interval. It should be pointed out that the use of MAF_{mean} for residues on plant surfaces is also supported by the new ESCORT 3 document (cf. [redacted], 2012[6]). A deterministic risk assessment is provided in the following.

[3] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[4] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

[5] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. [139 pp.].

[6] [redacted]; P. (2012): Rationale for harmonization of the multiple application factor (MAF) approach in ecotoxicological risk assessment. In: [redacted] (eds.): ESCORT 3: Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals. pp. 90-94. SETAC Press

Table 10.6- 2: Deterministic risk assessment based on the ER_{50} > 7 kg a.s./ha

Crop	Use pattern	Distance from field edge [m]	Drift [%]	MAF_m	PER [kg a.s./ha]	TER (Trigger = 5)
Pome fruits	3 x 3.6 kg a.s./ha (7 days interval)	3	11.01	2.0	0.793	> 8.8

From the calculations above, it is concluded that effects of the product on non-target terrestrial plants are not to be expected.

CP 10.6.1 Summary of screening data

Not necessary as guideline GLP studies for terrestrial non-target plants are available (see Section CP 10.6.2).

CP 10.6.2 Testing on non-target plants

Vegetative vigour

Report: KCP 10.6.2/01 [redacted]; 2000; M-199640-01-1
Title: Determination of effects on vegetative vigor of six plant species Aliette (R) 80WG
Report No.: C010801
Document No.: M-199640-01-1
Guideline(s): OECD Draft Guideline No. 208, Part B;
Equivalent to US EPA OPPTS Guideline No. 850.4150
Guideline deviation(s): Routine soil and water screening analyses for pesticides, PCBs and toxic metals were conducted using standard U.S. EPA procedures by GeoLabs, Inc., Braintree, Massachusetts. Physical characterization of the soil was performed by Agvise Labs, Northwoods, North Dakota. These data were not collected in accordance with Good Laboratory Practice procedures (i.e., no distinct protocol, Study Director, etc).
GLP/GEP: **yes**

Methods:
The study was conducted in a greenhouse. At test initiation, the 8 replicate pots of each treatment level for each species were grouped together and sprayed with the appropriate treatment solution. The plant species tested included two monocotyledons: onion (*Allium cepa*) and oat (*Avena sativa*), and four dicotyledons: cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*). The maximum application rate of ALIETTE 80WG was 80 kg a.s./ha. The test substance application volume was 1200 L/ha. Fosetyl-Al: 786 g/kg of test substance.
Effects on shoot dry weight were evaluated 21 d after the application of ALIETTE 80WG to the plant foliage. Tier 1 testing was conducted at the maximum application rate with all six species. Based on the adverse effects observed in cabbage and tomato, respectively, Tier 2 tests were conducted for these two species at 5, 10, 20, 40 and 80 kg a.s./ha.

Results:
The measured concentrations of fosetyl-Al for both applications were 92 and 94% of the nominal concentration. Due to effects on tomato, the following toxicity values were determined:

EC_{25} (tomato) = 8.6 kg a.s./ha
EC_{50} (tomato) > 80 kg a.s./ha
NOEC (tomato) = 5.0 kg a.s./ha

- **Comments (RMS):** acceptable

Further study information supplementing the original DAR summary:

Methods:
The test design was based on the OECD 208 B draft guideline[7] from July 2000. The test substance Aliette 80 WG (= Fosetyl-Al WG 80) was applied to leaves and other above-ground portion of the test plants at the 2 to 4 leaf stage. The plant species tested included two monocotyledons; onion (*Allium cepa*) and oat (*Avena sativa*), and four dicotyledons: cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*), giving a total of six plant families.
The test species cucumber, oat, onion and soybean were exposed to one test rate which was 80 kg a.s./ha. For the species cabbage and tomato a tier-2 test design was applied consisting of five test rates, i.e. 5.0, 10, 20, 40 and 80 kg a.s./ha. The test substance application volume was 200 L/ha for all species tested.
For each species eight replicate pots, each with five plants, were maintained for the control and each application rate. The pots were 13 cm tall with a diameter of 13 cm. Before and after application the pots were kept in a greenhouse. Water was provided by subirrigation.
The test species were assessed for morphological abnormalities and mortality on a weekly basis, shoot dry weight was determined at the end of the study period of 21 days.

Validity Criteria according to current test guidelines (OECD 227; July 2006[8])
Seedling emergence: At least 70% (in study: not documented but without influence on study outcome as more seeds were sown than needed and the surplus was later thinned out).
Plants in control do not exhibit visible phytotoxic effects (in study: no phytotoxic effect).
Plants in control exhibit only normal variation in growth and morphology (in study: no morphological abnormalities).
Mean plant survival in control is at least 90% for duration of study (in study: > 90%).

[7] OECD (Draft Document July 2000): Guideline for the testing of chemicals. Proposal for updating Guideline 208 "Terrestrial (Non-Target) Plant Test: 208 A: Seedling Emergence and Seedling Growth Test. 208 B: Vegetative Vigour Test.
[8] OECD Guideline 227 (July 2006): Guideline for the testing of chemicals, Terrestrial Plant Test: Vegetative vigour Test.

Findings

The measured concentrations of fosetyl-Al for both applications were 92 and 94% of the nominal concentration. In the following table effects on shoot dry weight of the single test species and corresponding endpoints, where available, are summarized.

Species	Summary of NOER, ER_{25} and ER_{50} values determined during vegetative vigor tests exposing six plant species to Fosetyl-Al WG 80			
	ER_{25} (kg a.s./ha)	ER_{50} (kg a.s./ha)	NOER (kg a.s./ha)	Difference (%)
Cabbage	>80	>80	80	0, 1, 5, 16, -11 [b]
Cucumber [a]	NA	NA	NA	9
Oat [a]	NA	NA	NA	-8
Onion [a]	NA	NA	NA	0
Soybean [a]	NA	NA	NA	4
Tomato	8.6	>80	5.0	18, 30, 26, 40, 33 [b]

a For Tier 1 tests, ER and NOER values were not calculated.
b Tier 2 testing was conducted at 5.0, 10, 20, 40 and 80 kg a.s./ha, respectively

Details of the observations of morphological abnormalities (at test termination) and mortality in this vegetative vigour study are provided in the following for each test species.

Nominal Application Rate (kg a.i./ha)	Cabbage Morphological Abnormalities[a] Nec In	Chl In	Phy In	Fru	Flo	Leaf Curl	Cumulative Mortality (#)[b] Day 21
Control	0.0	0.0	0.0	0	0	0	0
5.0	0.0	0.051	0.0	0	0	0	0
10	0.0	0.0	0.0	0	0	0	0
20	0.0	0.095	0.0	0	0	0	2
40	0.077	1.2	0.36	0	0	1	1
80	0.24	1.4	0.81	0	0	16	3

a Necrotic, chlorotic and phytotoxic indices are shaded and considered significant when ••1.0. The index ranges from 0 (lowest) to 4 (highest).
b Number of plants that died throughout test period.

Nec In = necrotic index
Chl In = chlorotic index
Phy In = phytotoxic index
Fru = fruit present
Flo = flowering present

Nominal Application Rate (kg a.i./ha)	Cucumber Morphological Abnormalities[a]						Cumulative Mortality (#)[b] Day 21
	Nec In	Chl In	Phy In	Fru	Flo	Leaf Curl	
Control	0.0	0.0	0.0	0	0	0	0
80	1.0	1.0	1.0	40	0	0	0

[a] Necrotic, chlorotic and phytotoxic indices are shaded and considered significant when • •1.0. The index ranges from 0 (lowest) to 4 (highest).
[b] Number of plants that died throughout test period.

Nec In = necrotic index
Chl In = chlorotic index
Phy In = phytotoxic index
Fru = fruit present
Flo = flowering present

Table 9. Observations of morphological abnormalities (at test termination) and mortalities (throughout the test period) for oat (*Avena sativa*) plants exposed to Aliette® 80WG during the vegetative vigor test.

Nominal Application Rate (kg a.i./ha)	Oat Morphological Abnormalities[a]						Cumulative Mortality (#)[b] Day 21
	Nec In	Chl In	Phy In	Fru	Flo	Leaf Curl	
Control	0.0	0.0	0.0	0	0	0	0
80	0.0	1.0	0.0	0	0	0	0

[a] Necrotic, chlorotic and phytotoxic indices are shaded and considered significant when • •1.0. The index ranges from 0 (lowest) to 4 (highest).
[b] Number of plants that died throughout test period.

Nec In = necrotic index
Chl In = chlorotic index
Phy In = phytotoxic index
Fru = fruit present
Flo = Flowering present

Nominal Application Rate (kg a.i./ha)	Onion Morphological Abnormalities[a]						Cumulative Mortality (#)[b] Day 21
	Nec In	Chl In	Phy In	Fru	Flo	Leaf Curl	
Control	0.0	0.0	0.0	0	0	0	0
80	0.05	0.0	0.05	0	0	0	0

[a] Necrotic, chlorotic and phytotoxic indices are shaded and considered significant when • •1.0. The index ranges from 0 (lowest) to 4 (highest).
[b] Number of plants that died throughout test period.

Nec In = necrotic index
Chl In = chlorotic index
Phy In = phytotoxic index
Fru = fruit present
Flo = flowering present

Nominal Application Rate (kg a.i./ha)	Soybean Morphological Abnormalities[a]						Cumulative Mortality (#)[b] Day 21
	Nec In	Chl In	Phy In	Fru	Flo	Leaf Curl	
Control	0.0	0.0	0.0	0	0	0	0
80	0.78	0.98	0.78	0	0	0	0

[a] Necrotic, chlorotic and phytotoxic indices are shaded and considered significant when • •1.0. The index ranges from 0 (lowest) to 4 (highest).
[b] Number of plants that died throughout test period.

Nec In = necrotic index
Chl In = chlorotic index
Phy In = phytotoxic index
Fru = fruit present
Flo = flowering present

Nominal Application Rate (kg a.i./ha)	Tomato Morphological Abnormalities[a]						Cumulative Mortality (#)[b] Day 21
	Nec In	Chl In	Phy In	Fru	Flo	Leaf Curl	
Control	0.0	0.0	0.0	0	0	0	0
5.0	0.0	0.0	0.0	0	0	0	0
10	0.0	0.0	0.0	0	0	0	0
20	0.0	0.0	0.0	0	0	0	0
40	0.0	1.0	0.0	0	0	0	0
80	1.0	1.0	1.0	0	0	0	0

[a] Necrotic, chlorotic and phytotoxic indices are shaded and considered significant when • •1.0. The index ranges from 0 (lowest) to 4 (highest).
[b] Number of plants that died throughout test period.

Nec In = necrotic index
Chl In = chlorotic index
Phy In = phytotoxic index
Fru = fruit present
Flo = flowering present

Conclusion:

The validity criteria of the current test guideline were fulfilled. No adverse effects > 50% were found in any species tested at the maximum application rate of 80 kg a.s./ha. Therefore, the ER_{50} is set > 80 kg a.s./ha.

Seedling emergence

Report:	KCP 10.6.2/02[redacted]; 2015; M-525769-01-1
Title:	Fosetyl-Al WG 80 percent w/w - Seedling emergence and seedling growth test
Report No.:	CEMS-6984
Document No.:	M-525769-01-1
Guideline(s):	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4100
Guideline deviation(s):	none
GLP/GEP:	**yes**

Objective:
The aim of this study was to determine potential effects on seedling emergence and early growth of higher plants following a single application of Fosetyl-aluminium WG 80 (Fosetyl-Al WG 80) to the soil surface directly after sowing. The endpoints of the study were emergence, plant survival, assessment of visual injury and shoot dry weight, in accordance with the requirements specified in the OECD Guideline 208 (July 2006) Terrestrial Plant Test, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test.

Material and Methods:
The test item was specified by batch no. EV36003889 and specification no. 102000024225 (analysed content of fosetyl-Al: 80.5% w/w). Control: Deionised water was used as the control treatment for the soil surface application.
A Tier I bioassay was carried out using 7 kg a.s./ha of Fosetyl-Al WG 80 at 200 L water/ha. Maize (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*), ryegrass (*Lolium perenne*), cucumber (*Cucumis sativus*), oilseed rape (*Brassica napus*), soybean (*Glycine max (G. soja)*), sugar beet (*Beta vulgaris*), sunflower (*Helianthus annuus*) and tomato (*Solanum lycopersicon*) were assessed for effects on emergence, survival and phytotoxicity for a test period of 21 days after 50% emergence in the control (DAE). After the final observation at 21 DAE the shoot dry weights were determined.
Glasshouse lighting was set to achieve a 16:8 hour light: dark cycle for the duration of the study.
Temperature ranged from 10.5-34.3 °C and humidity was between 16.2-85.1%.
There were two minor deviations from the recommended temperature range of 12 to 32 °C, but as the validity criteria for all species tested (except cucumber) were met, these were considered to have had no impact on the emergence, growth and health of the plants.

Results:
Confirmatory analysis of the concentration of fosetyl-Al in the spray solution of Fosetyl-Al WG 80 was conducted as a delegated phase. The measured levels of fosetyl-Al were within 80 to 120% of the nominal concentration (100.4 and 102.2%) and verified the concentration of fosetyl-Al in the spray solutions.

Species	Application rate (7 kg fosetyl-Al/ha)		
	Percentage difference relative to the control treatment at 21 days after 50% emergence in the control (21 DAE)		
	Emergence (%)	Survival (%)	Shoot dry weight (%)
Maize	-21.1	0.0	20.4
Oat	14.3	0.0	2.5
Onion	21.4	-5.9	-10.4
Ryegrass	5.9	0.0	8.3
Cucumber	-6.3	14.3	-8.0
Oilseed Rape	-33.3	-20.6	39.1
Soybean	11.1	-4.7	-0.2
Sugar Beet	0.0	0.0	10.0
Sunflower	0.0	0.0	-3.0
Tomato	5.6	0.0	4.7

- Negative figures indicate that there was a reduction when compared to the untreated control

This study has fulfilled the validity criteria stated in OECD Guideline 208; seedling emergence for all the species was at least 70%, the control plants showed no visible signs of phytotoxic injury to individual plants and all replicates maintained good growth throughout. Overall mean plant survival was at least 90% for the control plants, with the exception of cucumber at 88%, for the study duration. This was due to the slow growth and poor cotyledon development of one plant in replicate five and eight causing them to die after emergence. This was considered to be a natural variation and did not have an impact on the validity of the bioassay as remaining plants in the control grew well with good vigour.

Conclusions:
The soil surface application of Fosetyl-Al WG 80 at a rate of 7 kg a.s./ha to ten terrestrial plant species did not produce effects on emergence, survival and shoot dry weight reaching or exceeding the 50% threshold for further testing. The data for all plant species treated with the test item were not statistically significantly different compared to the control data.

CP 10.6.3 Extended laboratory studies on non-target plants

In view of the results presented in Section CP 10.6.2, no further studies are deemed necessary.

CP 10.6.4 Semi-field and field tests on non-target plants

In view of the results presented in Sections CP 10.6.2 and CP 10.6.3, no further studies are deemed necessary.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

No further tests on other terrestrial organism deem to be necessary due to the low to moderate acute and chronic ecotoxicity of Fosetyl-aluminium WG 80 as presented in Sections CP 10.1 to CP 10.6. Additionally, no public literature reference as evaluated in Document MCA, Section 9, reported on an adverse effect.

CP 10.8 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 WG 80 as presented in Sections CP 10.1 to CP 10.7, no monitoring of non-target organism is deemed to be necessary.