Bayer CropScience

Document Title

Summary of the ecotoxicological studies
Deltamethrin EW 15

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

2017-05-15

Author(s)

[redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted], [redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **From Bayer CropScience; or**
- **From other applicants once the period of data protection has expired**.

Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2015-11-04	Update of Point CP 10.3.1.5 and author list	M-480040-02-1
2016-05-20	Update of Point CP 10.3.2.4 inclusion of study M-408272-01-1 and the summary	M-480040-03-1
2017-05-15	Update of Point CP 10.3.2 with docuemnts M-557588-01-1 and M-588250-01-1	M-480040-04-1
2017-05-15	Update fo Point CP 10.2 inclusion of document M-583896-01-1	M-480040-05-1

[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

Additions to the document after the Completeness Check are highlighted in yellow. Content not necessary anymore is crossed out.

Table of Contents

CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Use pattern considered in this risk assessment

Table10- 1: Intended application pattern

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L/ha]	Maximum application rate, individual treatment (ranges) [g/ha] Deltamethrin
Sugarbeet	BBCH 10-49	1		0.5	7.5
Cauliflower	BBCH 10-49	2	14	0.5	7.5
Wheat	BBCH 10-83	2	14	0.42	6.25

Definition of the residue for risk assessment

Justification for the residue definition for risk assessment is provided in MCA Sec.7, Point 7.4.1 (environmental matrices) and MCA Sec.6 Point 6.7.1.

Table 10- 2: Definition of the residue for risk assessment

Compartment	Residue Definition
Soil	Deltamethrin (AE F032640) Br_2CA (AE F108565, *cis*) mPBacid (AE F109036)
Groundwater	Deltamethrin (AE F032640) Br_2CA (AE F108565, *cis*) mPBacid (AE F109036)
Surface water and sediment	Deltamethrin (AE F032640) Alpha-R-isomer of deltamethrin (AE F108569) Trans-isomer of deltamethrin (AE 0035073) 4'-OH-Deltamethrin (AE 0035082) Br_2CA (AE F108565, *cis*) BrCA isomer 1 (code not given) BrCA isomer 2 (code not given) Serinyl-BrCA (BCS-CW57835) mPBacid (AE F109036)
Air	Deltamethrin (AE F032640)
Plant	Deltamethrin (AE F032640)

Figure 10 - 1: Proposed degradation pathway of deltamethrin in soil (major metabolites are highlighted in bold writing)

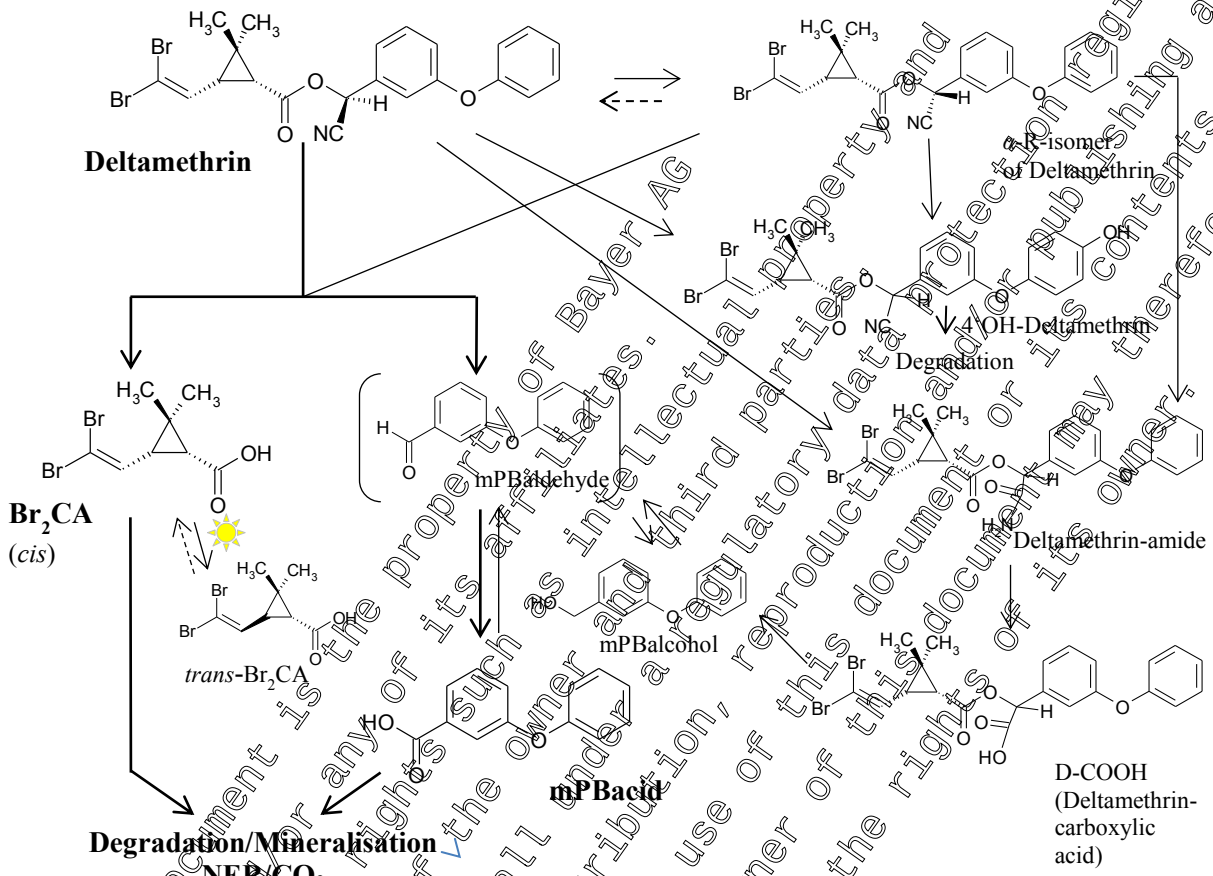
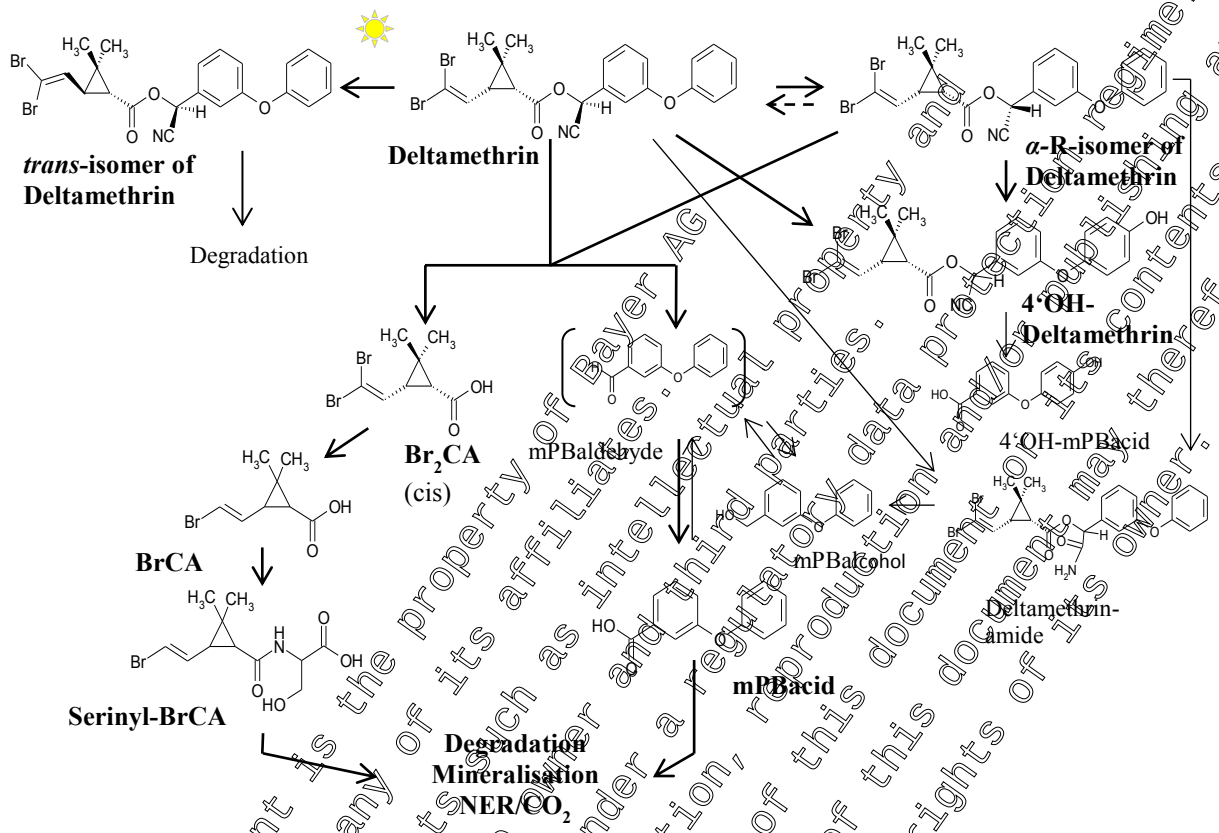


Figure 10 - 2: Proposed degradation pathway of deltamethrin in water and sediment



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).

CP 10.1.1 Effects on birds

Table 10.1.1- 1: Endpoints used in risk assessment

Test substance		Species	Endpoint	EU agreed endpoint (Review Report 6504/VI/99-final)	Reference
Deltamethrin	Acute risk assessment	Canary	LD_{50} 2000 mg as/kg bw	No	[redacted] (2013) M-444452-01-1 KCA 8.1.1.1/03
	Long-term risk assessment	Bobwhite quail	NOEL 450 ppm 55 mg as/kg bw/d[A]	Yes	[redacted] (1991) M-149397-01-1 KCA 8.1.1.1/01

[A] as reported in the original study report

Metabolites of deltamethrin

From toxicological studies performed in mammals there is no indication that the metabolites are more toxic than the active substance deltamethrin. For this reason and also considering animal welfare, no toxicity studies in birds with the metabolites were deemed necessary.

Table 10.1.1- 2: Relevant generic avian focal species for Tier 1 risk assessment (example)

Crop	Scenario	Generic focal species	Representative species	Shortcut value	
				Long-term RA based on RUD_m	acute RA based on RUD_{90}
Sugarbeet	**Late (summer/autumn)**	**Small granivorous bird "finch"**	**Linnet(*Carduelis cannabina*)**	**11.4**	**24.7**
	Early (spring) BBCH 10 - 19	**Small omnivorous bird "lark"**	**Woodlark (*Lulluba arborea*)**	**10.9**	**24.0**
	BBCH 10 - 19	Small insectivorous bird "wagtail"	Yellow wagtail (*Motacilla flava*)	5.9	10.9
	BBCH 20 – 49	Small insectivorous bird "wagtail"	Yellow wagtail (*Motacilla flava*)	2.8	7.7
	BBCH 20 – 49	**Small insectivorous bird "wagtail"**	**Yellow wagtail (*Motacilla flava*)**	**9.7**	**25.2**
Cauliflower (Leafy vegetables)	**BBCH 10 - 49**	**Small granivorous bird "finch"**	**Serin (*Serinus serinus*)**	**12.6**	**27.4**
	BBCH 10 - 49	**Small omnivorous bird "lark"**	**Woodlark (*Lulluba arborea*)**	**10.9**	**24.0**
	Leaf development BBCH 10 – 19	**Medium herbivorous /granivorous bird "pigeon"**	**Wood pigeon (*Columba palumbus*)**	**22.7**	**55.6**
	BBCH 10 - 19	**Small insectivorous bird "wagtail"**	**Yellow wagtail (*Motacilla flava*)**	**9.1**	**23.8**
	BBCH ≥ 20	**Small granivorous bird "finch"**	**Goldfinch (*Carduelis carduelis*)**	**11.4**	**24.6**
Wheat (Cereals)	**Late post emergence (May-June) BBCH 71-89**	**Small insectivorous bird "passerine"**	**Fan tailed warbler**	**22.4**	**57.6**
	Early (shoots) autumn-winter BBCH 10-29	**Large herbivorous bird "goose"**	**Pink-foot goose (*Anser brachyrhynchus*)**	**16.2**	**30.5**
	BBCH 10-29	**Small omnivorous bird "lark"**	**Woodlark (*Lulluba arborea*)**	**10.9**	**24.0**
	BBCH 30-39	Small omnivorous bird "lark"	Woodlark (*Lulluba arborea*)	5.4	12.0
	BBCH ≥ 40	Small omnivorous bird "lark"	Yellowhammer (*Emberiza citronella*)	3.3	7.2
	Late season – Seed heads	**Small granivorous/ insectivorous bird "bunting"**	**Yellowhammer (*Emberiza citronella)***	**12.5**	**27.0**

BOLD: Scenarios considered in risk assessment (only worst case for each species)

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 3: Tier 1 acute DDD and TER calculation for birds

Crop	Generic focal species	DDD			DDD	LD_{50} [mg/kg bw]	TER_A	Trigger
		Appl. rate [kg/ha]	SV_{90}	MAF_{90}				
Deltamethrin								
Sugarbeet	Small granivorous bird "finch"	0.0075	24.7	1	0.19	> 2000	> 10526	10
	Small omnivorous bird "lark"		24.0		0.18		> 11111	
	Small insectivorous bird "wagtail"		7.7		0.06		> 33333	
Cauliflower	Small granivorous bird "finch"	0.0075	27.4	1.2	0.25	> 2000	> 8000	10
	Small omnivorous bird "lark"		24.0		0.22		> 9091	
	Medium herbivorous/granivorous bird		55.6		0.50		> 4000	
	Small insectivorous bird "wagtail"		23.8		0.21		> 9524	
	Small granivorous bird "finch"		24.6		0.22		> 9091	
Wheat	Small insectivorous bird "passerine"	0.00625	57.6	1.2	0.43	> 2000	> 4651	10
	Large herbivorous bird "goose"		30.5		0.23		> 8696	
	Small omnivorous bird "lark"		24.0		0.18		> 11111	
	Small granivorous/ insectivorous bird "bunting"		27.0		0.2		> 10000	

The TER_A values calculated in the Tier 1 risk assessment for birds 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 from pools in leaf whorls

Table 10.1.1- 4: Tier 1 acute DDD and TER calculation for birds drinking contaminated water from pools in leaf whorls

Crop	DWR [L/kg bw/d]	PEC_{dw} [mg/L]	DDD [mg/kg bw/d]	LD_{50} [mg/kg bw]	TER_A	Trigger
Deltamethrin						
Cauliflower	0.46	7.5	3.45	> 2000	> 580	10

This evaluation confirms that the acute risk for birds from drinking water in leaf whorl puddles in cabbage that may contain residues from deltamethrin is acceptable.

LONG-TERM REPRODUCTIVE RISK ASSESSMENT

Table 10.1.1- 5 Tier 1 long-term DDD and TER calculation for birds

Compound / Crop	Generic focal species	DDD				DDD	NO(A)EL mg kg/bw/d	TER_{LT}	Trigger
		Appl. rate [kg/ha	SV_m	MAF_m	ftwa				
Deltamethrin									
Sugarbeet	Small granivorous bird "finch"	0.0075	11.4	1	0.53	0.05	55	1100	5
	Small omnivorous bird "lark"		10.9			0.04		1375	
	Small insectivorous bird "wagtail"		9.7			0.04		1375	
Cauliflower	Small granivorous bird "finch"	0.0075	12.6	1.4		0.07		786	5
	Small omnivorous bird "lark"		10.9			0.06		920	
	Medium herbivorous/granivorous bird "pigeon"		22.7			0.13		423	
	Small insectivorous bird "wagtail"		9.1			0.05		1100	
	Small granivorous bird "finch"		11.4			0.06		917	
Wheat	Small insectivorous bird "passerine"	0.00625	22.4	1.4		0.1		550	5
	Large herbivorous bird "goose"		16.2			0.08		688	
	Small omnivorous bird "lark"		10.9			0.05		1100	
	Small granivorous/ insectivorous bird "bunting"		12.3			0.06		917	

The TER_{LT} values calculated in the Tier 1 risk assessment for birds exceed the a-priori-acceptability trigger of 10 for all evaluated scenarios. Thus, the long-term risk to birds can be considered as low and acceptable without need for further, more realistic risk assessment.

Table 10.1.1- 6 Evaluation of potential concern for exposure of birds from drinking water (escape clause)

Crop	Koc [L/kg]	Application rate * MAF [g as/ha]	NO(A)EL [mg as/ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
Sugarbeet	10 240 000	7.5 × b	55	0.14	≤ 3000	No concern
Cauliflower		7.5 × 1.8[1]		0.25		No concern
Wheat		6.25 × 1.8[1]		0.20		No concern

[1] MAF based on a DT_{50} in soil of 54.8 days (geometric mean as used for PEC_{sw})

This evaluation confirms that the risk for birds from drinking water that may contain residues from deltamethrin is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

Table 10.1.1- 7 Log Pow values

Substance	log Pow	Reference
Deltamethrin	6.4 (pH 6.8)	M-437011-01-1 MCA 2.7/02
α-R isomer of deltamethrin	6.4 (pH 6.8)	M-437019-01-1 KCA 2.14 /16
trans-isomer of deltamethrin	6.3 (pH 6.8) 6.3 (pH 6.9)	M-435781-01-1 KCA 2.14 /19 M-436125-01-1 KCA 2.14 /22
Br_2CA*	3.1 (pH 5) 1.4 (pH 7) -0.1 (pH 9)	M-432956-01-1 KCA 2.14 /23
mPBacid*	2.8 (pH 5) 0.8 (pH 7) -0.4 (pH 9)	M-435852-01-1 KCA 2.14 /29
Serinyl-BrCA*	-0.2 (pH 5) -1.7 (pH 7) -2.1 (pH 9)	M-451650-01-1 KCA 2.14 /14
4'OH-Deltamethrin	4.5-4.6 (pH 5-9)	M-454807-01-1 KCA 2.14 /11

* No risk assessment of secondary poisoning will be performed as the log P_{OW} < 3 at ecologically relevant pH values.

Calculations in this section are performed for all compounds of the residue definition for soil and surface water (see Table 10- 2) with log P_{OW} > 3, employing toxicity values and BCF_{fish} as determined for the active substance deltamethrin, in order to demonstrate negligible overall risk.

Table 10.1.1- 8: Avian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic avian indicator species	Body weight [g]	Example	FIR/bw
Earthworm eater	100	Thrush	1.05
Fish eater	1000	Heron	0.159

Long-term DDD and TER calculation for earthworm-eating birds

Table 10.1.1- 9 Tier 1 long-term DDD and TER calculation for earthworm-eating birds

	Sugarbeet	Cauliflower	Wheat
Deltamethrin			
K_{OW}	2511887		
K_{OC} [mL/g]	10 240 000		
f_{OC}	0.02		
BCF_{worm}	0.147		
PEC_{soil} (twa, 21 d) [mg/kg]	0.00775	0.01424	0.00985
PEC_{worm} [mg/kg]	0.001	0.002	0.001
FIR/bw	1.05	1.05	1.05
DDD [mg/kg bw/d]	0.001	0.002	0.001
NO(A)EL [mg/kg bw/d]	55		
TER_{LT}	55000	27500	55000
Trigger	5	5	5

Long-term DDD and TER calculation for fish-eating birds

Table 10.1.1- 10 Tier 1 long-term DDD and TER calculation for fish-eating birds

Sugarbeet				
Substance	**Deltamethrin**	**α-R isomer of deltamethrin**	***trans*-isomer of deltamethrin**	**4'OH-Deltamethrin**
BCF_{fish}	1400			
PEC_{SW} (twa, 21 d)[mg/L]	< 0.00001	< 0.00001	0.00001	< 0.00001
PEC_{fish} [mg/kg]	< 0.014	< 0.014	0.014	< 0.014
FIR/bw	0.159	0.159	0.159	0.159
DDD [mg/kg bw/d]	< 0.002	< 0.002	0.002	< 0.002
NO(A)EL [mg/kg bw/d]	55			
TER_{LT}	27000	27000	27000	> 27000
Trigger	5	5	5	5

Cauliflower				
Substance	**Deltamethrin**	**α-R isomer of deltamethrin**	**trans-isomer of deltamethrin**	**4'OH-Deltamethrin**
BCF_{fish}	1400			
PEC_{SW} (twa, 21 d)[mg/L]	< 0.00001	< 0.00001	0.00002	< 0.00001
PEC_{fish} [mg/kg]	< 0.014	< 0.014	0.028	< 0.014
FIR/bw	0.159	0.159	0.159	0.159
DDD [mg/kg bw/d]	< 0.002	< 0.002	0.004	< 0.002
NO(A)EL [mg/kg bw/d]	55			
TER_{LT}	> 27500	> 27500	13750	> 27500
Trigger	5	5	5	5

Wheat				
Substance	**Deltamethrin**	**α-R isomer of deltamethrin**	**trans-isomer of deltamethrin**	**4'OH-Deltamethrin**
BCF_{fish}	1400			
PEC_{SW} (twa, 21 d)[mg/L]	< 0.00001	< 0.00001	0.00002	< 0.00001
PEC_{fish} [mg/kg]	< 0.014	< 0.014	0.028	< 0.014
FIR/bw	0.159	0.159	0.159	0.159
DDD [mg/kg bw/d]	< 0.002	< 0.002	0.004	< 0.002
NO(A)EL [mg/kg bw/d]	55			
TER_{LT}	> 27500	> 27500	13750	> 27500
Trigger	5	5	5	5

The TER values for deltamethrin, the α-R isomer and the trans-isomer of deltamethrin as well as for 4'OH-Deltamethrin are above the trigger of concern of 5, indicating no risk from secondary poisoning for earthworm- and fish-eating birds.

CP 10.1.1.1 Acute oral toxicity

No new studies were required.

CP 10.1.1.2 Higher tier data on birds

In view of the results presented above, 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		Species	Endpoint		EU agreed endpoint (Review Report 6504/VI/99-final)	Reference
Deltamethrin	Acute risk assessment	Rat	LD_{50}	87 mg as/kg bw	Yes	M-139700-01-1 KCA 5.2.1/04
	Long-term risk assessment	Rat	NO(A)ED	4.2[A] mg as/kg bw/d	No	M-149348-01-1 KCA 5.6.1/01

[A] The justification for the use of this endpoint is presented under MCA 8.1.1.2

Metabolites of deltamethrin

For mammals, one study for acute oral toxicity in rats was performed to evaluate toxic effects of the deltamethrin metabolite Br_2CA. The metabolite Br_2CA exhibited no toxicity to rats by using plant oil as carrier, and thus, the study is comparable to that conducted with deltamethrin by [redacted] (1996; M-139700-01-1), which revealed the lowest acute endpoints for deltamethrin.
Since the metabolite proofed to be less toxic than the parent compound, explicit TER values for Br_2CA were not calculated as the risk assessment is considered to be covered by that of the parent compound.

Table 10.1.2- 2 Relevant generic focal species for Tier 1 risk assessment

Crop	Scenario	Generic focal species	Representative species	Shortcut value	
				Long-term RA based on RUD_m	acute RA based on RUD_{90}
Sugarbeet	**BBCH 10 - 19**	**Small insectivorous mammal "shrew"**	**Common shrew (*Sorex araneus*)**	**4.2**	**7.6**
	BBCH ≥ 20	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	1.9	5.4
	BBCH ≥ 40	**Small herbivorous mammal "vole"**	**Common vole (*Microtus arvalis*)**	**18.1**	**34.1**
	BBCH 10 - 39	**Large herbivorous mammal**	**Rabbit (*Oryctolagus cuniculus*)**	**14.3**	**35.1**
	BBCH ≥ 40	Large herbivorous mammal "lagomorph"	Rabbit (*Oryctolagus cuniculus*)	3.6	8.8
	BBCH 10 - 39	**Small omnivorous mammal "mouse"**	**Woodmouse (*Apodemus sylvaticus*)**	**7.8**	**17.2**
	BBCH ≥ 40	Small omnivorous mammal "mouse"	Woodmouse (*Apodemus sylvaticus*)	1.9	4.3
Cauliflower (Leafy vegetables)	**BBCH 10 - 19**	**Small insectivorous mammal "shrew"**	**Common shrew (*Sorex araneus*)**	**4.2**	**7.6**
	BBCH ≥ 20	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	1.9	5.4
	BBCH 40 - 49	**Small herbivorous mammal "vole"**	**Common vole (*Microtus arvalis*)**	**72.3**	**136.4**
	All season	**Large herbivorous mammal**	**Rabbit (*Oryctolagus cuniculus*)**	**14.3**	**35.1**
	BBCH 10 - 49	**Small omnivorous mammal "mouse"**	**Woodmouse (*Apodemus sylvaticus*)**	**7.8**	**17.2**
Wheat (Cereals)	**BBCH 10 - 19**	**Small insectivorous mammal "shrew"**	**Common shrew (*Sorex araneus*)**	**4.2**	**7.6**
	BBCH ≥ 20	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	1.9	5.4
	BBCH ≥ 40	**Small herbivorous mammal "vole"**	**Common vole (*Microtus arvalis*)**	**21.7**	**40.9**
	Early (shoots)	**Large herbivorous mammal**	**Rabbit (*Oryctolagus cuniculus*)**	**22.3**	**42.1**
	BBCH 10 - 29	**Small omnivorous mammal "mouse"**	**Woodmouse (*Apodemus sylvaticus*)**	**7.8**	**17.2**
	BBCH 30 – 39	Small omnivorous mammal "mouse"	Woodmouse (*Apodemus sylvaticus*)	3.9	8.6
	BBCH ≥ 40	Small omnivorous mammal "mouse"	Woodmouse (*Apodemus sylvaticus*)	2.3	5.2

BOLD: considered in risk assessment (only worst case for each species)

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 3 Tier 1 acute DDD and TER calculation for mammals

Crop	Generic focal species	DDD			DDD	LD_{50} [mg/kg bw]	TER_A	Trigger
		Appl. rate [kg/ha]	SV_{90}	MAF_{90}				
Deltamethrin								
Sugarbeet	Small insectivorous mammal "shrew"	0.0075	7.6	1	0.06	87	1450	10
	Small herbivorous mammal "vole"		34.1		0.26		335	
	Large herbivorous mammal "lagomorph		35.1		0.26		335	
	Small omnivorous mammal "mouse"		17.2		0.13		669	
Cauliflower	Small insectivorous mammal "shrew"	0.0075	7.6	1.2	0.07		1243	
	Small herbivorous mammal "vole"		136.4		1.23		71	
	Large herbivorous mammal "lagomorph"		35.1		0.32		272	
	Small omnivorous mammal "mouse"		17.2		0.15		580	
Wheat	Small insectivorous mammal "shrew"	0.00625	7.6	1.2	0.06		1450	
	Small herbivorous mammal "vole"		40.9		0.31		281	
	Large herbivorous mammal "lagomorph"		42.1		0.32		272	
	Small omnivorous mammal "mouse"		17.2		0.13		669	

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

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.2- 4 Tier 1 long-term DDD and TER calculation for mammals

Crop	Generic focal species	DDD				DDD	NO(A)EL mg kg/bw/d	TER_{LT}	Trigger
		Appl. rate [kg/ha	SV_m	MAF_m	f_{twa}				
Deltamethrin									
Sugarbeet	Small insectivorous mammal "shrew"	0.0075	4.2	1	1	0.02	4.2	210	5
	Small herbivorous mammal "vole"		18.1			0.02		210	
	Large herbivorous mammal "lagomorph		14.3			0.06		70	
	Small omnivorous mammal "mouse"		7.8			0.03		140	
Cauliflower	Small insectivorous mammal "shrew"	0.0075	4.2	1.4	0.53	0.02	4.2	210	5
	Small herbivorous mammal "vole"		72.3			0.040		11	
	Large herbivorous mammal "lagomorph"		14.3			0.08		53	
	Small omnivorous mammal "mouse"		7.8			0.04		105	
Wheat	Small insectivorous mammal "shrew"	0.00625	4.2	1.4	1	0.02		210	5
	Small herbivorous mammal "vole"		21.7			0.10		42	
	Large herbivorous mammal "lagomorph"		22.3			0.10		42	
	Small omnivorous mammal "mouse"		7.8			0.04		105	

The TER_{LT} values calculated in the Tier 1 risk assessment for mammals exceed the a-priori-acceptability trigger of 5 for all evaluated scenarios. Thus, the long-term risk to 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- 5 Evaluation of potential concern for exposure of mammals from drinking water

Crop	Koc [L/kg]	Application rate * MAF [g as/ha]	NO(A)EL [mg as/ kg bw/d]	Ratio (Application rate * MAF) / NO(A)EL	"Escape clause" No concern if ratio	Conclusion
Sugarbeet	10 240 000	7.5 × 1	4.2	1.8	≤ 3000	No concern
Cauliflower		7.5 × 1.8[1]		3.2		No concern
Wheat		6.25 × 1.8[1]		2.7		No concern

[1] MAF based on a DT_{50} in soil of 54.8 days (geometric mean as used for PEC_{sw})

This evaluation confirms that the risk for mammals from drinking water that may contain residues from deltamethrin is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

As outlined in Point 10.1.1 a risk assessment of secondary poisoning has be performed for all compounds of the residue definition for soil and surface water (see Table 10- 2) with log P_{OW} > 3, employing toxicity values and BCF_{fish} as determined for the active substance deltamethrin, in order to demonstrate negligible overall risk.

Table 10.1.2- 6 Mammalian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic focal species	Body weight [g]	Example	FIR/bw
Earthworm eater	10	Common shrew	1.28
Fish eater	3000	Otter	0.142

Long-term DDD and TER calculation for earthworm-eating mammals

Table 10.1.2- 7 Tier 1 long-term ETE and TER calculation for earthworm eating mammals

	Sugarbeet	Cauliflower	Wheat
Substance	Deltamethrin		
PEC_{worm} [mg/kg]	0.001	0.002	0.001
FIR/bw	1.28	1.28	1.28
DDD [mg/kg bw/d]	0.001	0.003	0.001
NO(A)EL [mg/kg bw/d]	4.2		
TER_{LT}	4200	1400	4200
Trigger	5	5	5

Long-term toxicity exposure ratio for fish-eating mammals

Table 10.1.2- 8 Tier 1 long-term ETE and TER calculation for fish eating mammals

Sugarbeet				
Substance	**Deltamethrin**	**α-R isomer of deltamethrin**	***trans*-isomer of deltamethrin**	**4'OH-Deltamethrin**
PEC_{fish} [mg/kg]	< 0.014	< 0.014	0.014	< 0.014
FIR/bw	0.142	0.142	0.142	0.142
DDD [mg/kg bw/d]	0.002	< 0.002	0.002	< 0.002
NO(A)EL [mg/kg bw/d]	4.2			
TER_{LT}	> 2100	2100	2100	> 2100
Trigger	5	5	5	5

Cauliflower				
Substance	**Deltamethrin**	**α-R isomer of deltamethrin**	***trans*-isomer of deltamethrin**	**4'OH-Deltamethrin**
PEC_{fish} [mg/kg]	< 0.014	< 0.014	0.028	< 0.014
FIR/bw	0.142	0.142	0.142	0.142
DDD [mg/kg bw/d]	< 0.002	< 0.002	0.004	< 0.002
NO(A)EL [mg/kg bw/d]	4.2			
TER_{LT}	> 2100	> 2100	2100	> 2100
Trigger	5	5	5	5

Wheat				
Substance	**Deltamethrin**	**α-R isomer of deltamethrin**	***trans*-isomer of deltamethrin**	**4'OH-Deltamethrin**
PEC_{fish} [mg/kg]	< 0.014	< 0.014	0.028	< 0.014
FIR/bw	0.142	0.142	0.142	0.142
DDD [mg/kg bw/d]	< 0.002	< 0.002	0.004	< 0.002
NO(A)EL [mg/kg bw/d]	4.2			
TER_{LT}	> 2100	> 2100	2100	> 2100
Trigger	5	5	5	5

CP 10.1.2.1 Acute oral toxicity to mammals

Please refer to MCP 7.1.1 where a summary of the formulation study (rat, acute oral; [redacted], 2000, M-197188-01-1) is presented.
The oral LD50 of the formulation was found to be higher than 2000 mg/kg b.w. in Sprague-Dawley rats.

CP 10.1.2.2 Higher tier data on mammals

In view of the results presented above, no further studies were necessary.

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

Since deltamethrin is of low toxicity in birds and laboratory rodents, no risk for reptiles and amphibians is to be expected.

CP 10.2 Effects on aquatic organisms

Deltamethrin EW15

Aquatic studies were conducted with the representative formulation Deltamethrin EW 15. Summaries of these studies are provided under point CP 10.2.1, while Table 10.2-1 gives an overview of the resulting endpoints.

Table 10.2- 1: Toxicity of the formulated product deltamethrin EW 15 to aquatic organisms

Test substance	Test species	Endpoint		Reference
Deltamethrin EW15	Fish, acute *Oncorhynchus mykiss*	LC_{50}	14.4 µg prod./L (mm)	[redacted]. (2000) M-197428-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	1.33 µg prod./L (mm)	[redacted]. (2000) M-197398-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	0.7 µg prod./L (mm)	[redacted] (2013) M-470588-01-1
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50}	8140 µg prod./L (mm)	[redacted]. (2000) M-197387-01-1
	Algae, growth inhibition *Pseudokirchneriella subcapitata*	E_rC_{50}	5350 µg prod./L (mm)	[redacted] (2011) M-413217-01-1

Ecotoxicological endpoints of the formulation Deltamethrin EW15 reflect the toxicity of the active substance deltamethrin. Minor deviations are considered normal due to the natural variability of biological systems and do not indicate a higher toxicity of the formulated product. Hence, it is justified

to base the aquatic risk assessment on endpoints derived from studies with the active substance deltamethrin.

Enquiries concerning the active substance studies and the studies with the formulation have been answered according to document M-583896-01-1.

Active substance deltamethrin

Endpoints from aquatic studies with the active substance deltamethrin are summarized in Table 10.2-2.

Table 10.2- 2: Toxicity of the active substance deltamethrin to aquatic organisms

Test organism	Test system	Endpoint [µg a.s./L]		EU agreed endpoint (Review Report 6504/VI/99-final)	Reference
Acute toxicity to fish					
Oncorhynchus mykiss (rainbow trout)	Static 96 h	LC_{50}	0.91 (nom)	Yes	[redacted] (1986) M-149417-01-1 KCA 8.2.1/02
Oncorhynchus mykiss (rainbow trout)	Flow-through 96 h	**LC_{50}**	**0.15 (mm)**	New endpoint suggested, derived from a study with chemical analysis	[redacted] (1990) M-135553-01-1 KCA 8.2.1/03
Lepomis macrochirus (bluegill sunfish)	Static 96 h	LC_{50}	1.4 (nom)	Already evaluated on EU level	[redacted] (1986) M-149416-01-1 KCA 8.2.1/01
Cyprinodon variegatus (sheepshead minnow)	Flow-through 96 h	LC_{50}	0.48 (mm)	Marine species not considered in last EU review	[redacted] (1990) M-135536-01-1 KCA 8.2.1/04
Chronic toxicity to fish					
Oncorhynchus mykiss (rainbow trout) Prolonged toxicity	Juvenile growth, flow-through 28 d	NOEC	< 0.032 (mm)	Yes, but lower endpoint available	[redacted] (1990) M-135553-01-1 KCA 8.2.2.1/01
Pimephales promelas (fathead minnow) Early Life Stage (ELS)	Early Life Stage, flow-through 36 d	NOEC	0.022 (mm)	Already evaluated on EU level	[redacted] (1991) M-149413-01-1 KCA 8.2.2/01
Cyprinodon variegatus (sheepshead minnow) Early Life Stage (ELS)	Early Life Stage, flow-through 35 d	NOEC	0.024 (mm)	New study	[redacted] (2012) M-439783-01-1 KCA 8.2.2.1/02
Pimephales promelas (fathead minnow) Fish full life cycle	Life cycle test, flow-through 260 d	**NOEC**	**0.017 (mm)**	Already evaluated on EU level; lowest chronic endpoint for fish – should be considered for risk assessment	[redacted] (1993) M-149454-01-1 KCA 8.2.2.2/01
Acute toxicity to invertebrates					
Daphnia magna (water flea)	Flow-through 48 h	EC_{50}	0.56 (mm)	Yes, but new study available	[redacted] (1999) M-187113-01-1 KCA 8.2.4.1/02

Test organism	Test system	Endpoint [µg a.s./L]		EU agreed endpoint (Review Report 6504/VI/99-final)	Reference
Daphnia magna (water flea)	Static-renewal 48 h	EC_{50}	0.0131 (mm)	New study resulting in lower endpoint – to be considered in RA	[redacted] (2014) M-474181-01-1 KCA 8.2.4.1/03
Hyalella azteca	Flow-through, 96 h, water only	LC_{50}	**0.00017 (mm)**	New study	[redacted] (2013) M-461147-01-1 KCA 8.2.4.2/01
Americamysis bahia (mysid shrimp)	Static-renewal 96 h	LC_{50}	0.0037 (mm)	Marine species not considered in last EU review	[redacted] (1991) M-149478-01-1 KCA 8.2.4.2/02
Chronic toxicity to invertebrates					
Daphnia magna (water flea)	Flow-through 21 d	NOEC	**0.0041 (mm)**	Yes	[redacted] (1991) M-174935-01-1 KCA 8.2.5.1/01
Americamysis bahia (mysid shrimp)	Flow-through 35 d	NOEC	**0.00073 (mm)**	New study	[redacted] (2013) M-437923-01-1 KCA 8.2.5.2/01
Chronic toxicity to sediment dwelling organisms					
Chironomus riparius (chironomid)	Static, 28 d, spiked water	NOEC	**0.010 (nom)**	Yes	[redacted] (1998) M-152560-01-1 KCA 8.2.5.3/01
Chironomus riparius (chironomid)	Static, 28 d, spiked sediment	EC_{10}	**7.5 µg a.s./kg dw sed (nom)**	New study	[redacted] (2012) M-425202-01-1 KCA 8.2.5.4/01
Chironomus dilutus (chironomid)	Flow-through, 63 d, spiked sediment	NOEC	**1.5 µg a.s./kg sed (mm)**	New study	[redacted] (2013) M-466314-01-1 KCA 8.2.5.4/02
Algae/Plant					
Pseudokirchneriella subcapitata	Static 96 h	E_rC_{50}	> 9100 (im)	Study evaluated on EU level but enpoint considered uncertain	[redacted] (1990) M-149388-01-1 KCA 8.2.6/01
Navicula pelliculosa	Static 96 h	E_rC_{50} E_bC_{50}	>3.1 (im) >3.1 (im)	New study	[redacted] (2013) M-468384-01-1 KCA 8.2.6.2/01
Anabaena flos-aquae	Static 96 h	E_rC_{50} E_bC_{50}	>3.6 (im) >3.6 (im)	New study	[redacted] (2013) M-468386-01-1 KCA 8.2.6.2/02
Skeletonema costatum	Static 96 h	E_rC_{50} E_bC_{50}	>3.4 (im) >3.4 (im)	New study	[redacted] (2013) M-468465-01-1 KCA 8.2.6.2/03
Lemna gibba	Static-renewal 7 d	E_rC_{50} E_bC_{50}	>0.779 (im) >0.779(im)	New study	[redacted] (2012) M-439085-01-1 KCA 8.2.7/01

Metabolites of deltamethrin

To complete the aquatic risk assessment, additional studies were conducted with the metabolites of deltamethrin, which are relevant for the aquatic environment (refer to Table 10-2 for residue definition in surface water and sediment). Table 10.2-3 gives an overview of the respective endpoints. Summaries of these studies are provided in the MCA document.

Table 10.2- 3 Toxicity of deltamethrin metabolites to aquatic organisms

Test substance	Test species	Endpoint	Reference
alpha-R-isomer of deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50} 16.2 µg/L (mm)*	[redacted] (2014) M-473954-01-1 KCA 8.2.1/05
	Invertebrate, acute *Daphnia magna*	EC_{50} 0.0366 µg/L (mm)*	[redacted] (2014) M-474118-01-1 KCA 8.2.4.1/04
trans-isomer of deltamethrin	Fish, acute *Oncorhynchus mykiss*	LC_{50} 0.239 µg/L (mm)*	[redacted] (2013) M-473731-01-1 KCA 8.2.1/06
	Invertebrate, acute *Daphnia magna*	EC_{50} 0.069 µg/L (mm)*	[redacted] (2014) M-473835-01-1 KCA 8.2.4.1/05
4'OH-deltamethrin (BCS-BY84407)	Fish, acute *Oncorhynchus mykiss*	LC_{50} 2.99 µg/L (mm)	[redacted] (2013) M-473195-01-1 KCA 8.2.1/07
	Invertebrate, acute *Daphnia magna*	EC_{50} 670 µg/L (mm)	[redacted] (2013) M-466317-01-1 KCA 8.2.4.1/06
Br_2CA (AE F108565)	Fish, acute *Oncorhynchus mykiss*	LC_{50} 100000 µg/L (nom)	[redacted] & [redacted] (2001) M-199816-01-2 KCA 8.2.1/08
	Invertebrate, acute *Daphnia magna*	EC_{50} 100000 µg/L (nom)	[redacted] & [redacted] (2001) M-199793-01-2 KCA 8.2.4.1/07
Serinyl-BrCA (BCS-CW57835)	Invertebrate, acute *Daphnia magna*	EC_{50} 35300 µg/L (mm)	[redacted] (2013) M-465372-01-1 KCA 8.2.4.1/08
mPBaldehyde** (AE F114152)	Invertebrate, acute *Daphnia magna*	EC_{50} 162 µg/L (mm)	[redacted] (2010) M-386854-01-1 KCA 8.2.4.1/09
mPBacid	Fish, acute *Oncorhynchus mykiss*	LC_{50} >3300 µg/L	[redacted] (1981) BL/B/2038 Syngenta number CGA55186/0707 KCA 8.2.1/09 M-479954-01-1 (Letter of Access)
	Invertebrate, acute *Daphnia magna*	EC_{50} 85000 µg/L	[redacted] (1983) Syngenta number CGA55186/0721 KCA 8.2.4.1/10 M-479954-01-1 (Letter of Access)

nom = nominal, mm = mean measured

* Results from the studies with the alpha-R-isomer and the trans-isomer of deltamethrin are not suitable for the use in aquatic risk assessments. An explanation is provided below.

** mPBaldehyde is only a minor metabolite and will not be considered in the risk assessment.

Aquatic risk assessment for metabolites

Results from the aquatic toxicity studies with the **alpha-R-isomer and the trans-isomer of deltamethrin** are not suitable for the use in aquatic risk assessments. In all four studies, the parent compound deltamethrin was also detected at concentrations, which are lethal to fish and *Daphnia*. Therefore it is expected, that deltamethrin contributed significantly to the toxic effects observed in these studies. In a conservative approach, endpoints were derived based on the mean measured concentrations of the respective metabolite only. These endpoints most likely overestimate the actual toxicity of the alpha-R-isomer and the trans-isomer of deltamethrin, as they do not consider the effects caused by the presence of the parent compound deltamethrin. The available studies do not allow for a definite determination of the metabolite toxicity, and are therefore not considered adequate for a risk assessment. Nevertheless, these worst-case endpoints clearly demonstrate that neither the alpha-R-isomer nor the trans-isomer of deltamethrin is more toxic to aquatic organisms than the parent compound itself.

The alpha-R-isomer and the trans-isomer of deltamethrin are formed rapidly from deltamethrin in the aquatic environment. As demonstrated in the laboratory studies with the metabolites, both isomers also re-isomerize into the parent compound deltamethrin.

Drift is the only relevant entry pathway of deltamethrin into surface water bodies, with subsequent formation of the metabolites alpha-R-isomer and trans-isomer of deltamethrin. Significant entry of the parent or the two metabolites after formation in soil via runoff or drainage can be excluded. This means, that the maximum PECsw value for deltamethrin occurs directly after application due to drift entry. PECsw values for a single application, i.e. using the highest drift rate, are considered for the TER calculations as a worst case. This initial PECsw for the parent compound deltamethrin covers also the two isomers, as – even if the metabolites are formed quickly – the sum of all three compounds cannot exceed this value. Therefore, the risk assessment conducted for deltamethrin covers the two isomers as well.

The higher tier risk assessments for aquatic invertebrates and fish are based on endpoints from mesocosm studies taking into account nominal deltamethrin concentrations.

As neither the alpha-R-isomer nor the trans-isomer of deltamethrin are more toxic than deltamethrin, it can be concluded that the refined aquatic risk assessment for the parent compound based on nominal endpoints from mesocosm studies and initial drift PECsw values for deltamethrin, covers also the two metabolites alpha-R- and trans-isomer of deltamethrin.

The metabolite **BrCA** was identified as major metabolite and needs to be addressed in the aquatic risk assessment. BrCA is formed from Br_2CA via elimination of a brome atom. Other than that, the two metabolites are identical. Br_2CA showed no toxicity to aquatic organisms in acute studies, with an LC_{50} of 100 mg/L for fish and an EC_{50} > 100 mg/L for *Daphnia*, respectively. Therefore, it is not expected that the metabolite BrCA poses a risk to aquatic organisms. No studies were conducted for this metabolite.

Acute TER calculations are provided for the metabolites **4'OH-deltamethrin**, **Br_2CA**, **Serinyl-BrCA** and **mPBacid**.

Refined risk assessment for fish

The Tier 1 risk assessments for fish are based on endpoints from laboratory studies, i.e. an acute LC_{50} of 0.15 µg a.s./L and a chronic NOEC of 0.017 µg a.s./L, respectively. However, both endpoints were derived under flow-through conditions, where the test concentration of the substance is artificially

maintained over the test period (i.e. 96 h for acute testing, 260 d in the FFLC study). Due to this study design, the results considerably overestimate the toxicity of deltamethrin to fish under realistic exposure conditions.

Due to its strong adsorption to organic matter, drift is considered the only relevant entry pathway for deltamethrin to surface water bodies. Continuous exposure, e.g. via drainage or repeated runoff events, are not expected for this compound. Moreover, deltamethrin dissipates rapidly in natural water bodies (with a dissipation DT_{50} in water of1 day), especially due to adsorption to particulate matter, sediment and macrophytes, which reduces the bioavailability of the substance under natural conditions very fast.

To obtain data on potential effects of deltamethrin on fish in the environment, a higher tier study was performed in outdoor enclosures simulating reasonable worst-case conditions ([redacted], 2005, M-256605-01-1). The representative formulation Deltamethrin EW15 was applied onto the water surface three times at a 7-day interval simulating spray drift. Juvenile fish were exposed to nominal deltamethrin concentrations of 125, 250, 500 and 1000 ng a.s./L for 21 days.

Compared to surface waters in agricultural areas, the oligo-mesotrophic microcosm system represents a worst case regarding nutrient content of water, and consequently growth of algae and macrophytes. Dissipation of deltamethrin is expected to be even higher in relevant water bodies in agricultural landscapes. Also, the test design considered three applications of deltamethrin at a seven-day interval, whereas only 1-2 applications are intended in sugarbeet, cauliflower and wheat. Therefore, the exposure profile of this study can be considered conservative.

The study was conducted with Rainbow trout (*O. mykiss*), which was identified as the most sensitive fish species in acute laboratory studies (see Table 10.2-2). For deltamethrin, growth turned out to be the most sensitive endpoint in the chronic fish studies with Fathead minnow (*P. promelas*) (Early Life Stage (ELS) and Fish Full Life Cycle (FFLC) study). For this reason, in addition to survival, growth was also assessed in the microcosm study.

No treatment related effects on survival were observed up to the highest treatment level of 1000 ng a.s./L. Furthermore, the most sensitive endpoint growth (measured as length, wet weight, dry weight, growth of length and weight) from the fathead minnow studies was not affected even at the highest test concentration. Although slight symptoms (swimming behaviour, coughing) occurred for a few hours on day 1 after the first application at 500 ng a.s./L, the overall NOEC of this study on rainbow trout has been set at 500 ng a.s./L. Considering the fast recovery of the initially observed symptoms, **the NOEAEC was set at 1000 ng a.s./L**.

To summarise, this outdoor microcosm study was performed:

- under realistic exposure conditions in a natural freshwater community,
- taking into account three applications of deltamethrin at a seven-day interval,
- with the most sensitive fish species Rainbow trout

At the NOEAEC of this study, no adverse effects on the overall most sensitive endpoint (growth in weight according to the results of the laboratory ELS and FFLC studies) were observed. For these reasons, the use of the NOEAEC and the chronic assessment factor of 10 seems adequate for the final risk assessment for fish, resulting in a Regulatory Acceptable Concentration (RAC) of 100 ng a.s./L. As long-term exposure of fish to deltamethrin is not expected in the environment, the endpoint from this 21-day study is considered appropriate to cover both, the acute and chronic risk of deltamethrin to fish.

This conclusion is in line with and further supported by other semi-field studies, which were already evaluated for the last Annex I inclusion. A brief summary of the key findings of these studies is provided in the following:

- Experimental ponds ([redacted] 1981; M-095315-01-1; Decis EC25 baseline dossier, KCP 10.2.2/01): No mortality was observed in Roach (*Rutilus rutilus*) and Crucian carp (*Carassius carassius*) at nominal concentration of 1 µg a.s./L from overspray at 10 g a.s./ha to 1 m deep water (≈3 g a.s./ha to a standard 0.3 m deep water). Severe mortality was observed in fish at a nominal concentration of 5 µg/L from overspray at 50 g/ha to 1 m deep water (≈ 15 g a.s./ha to a 0.3 m deep water) after 14 days of observation.
- Microcosms ([redacted] 1991; M-136691-01-1; Decis EC25 baseline dossier, KCP 9.2.5/01 No adverse effects were observed in Fathead minnow (*Pimephales promelas*) at a nominal concentration of 2.2 µg a.s./L from overspray at 20 g a.s./ha to 0.9 m deep water (≈ 6.7 g a.s./ha to a 0.3 m deep water) after 7 days of observation.
- Mesocosms ([redacted], 1985; M-113322-01-1; KCA 8.2.8/02): No mortality was observed in Fathead minnow *(Pimephales promelas)* at a nominal concentration of 3.2 µg a.s./L from application of 10 g a.s./ha below the surface of a 0.9 m deep water body (≈ 6 g a.s./ha to a 0.3 m deep water) up to 112 days post-treatment.
- Mesocosm study on tralomethrin ([redacted] doc. M-136731-01-1 and M-152210-01-1; KCA 8.2/01 and KCA 8.2/02), which is rapidly transformed into deltamethrin and which is very similar to this compound in terms of mode of action and toxicity (in the last EU peer review, it was agreed, that the data on fish from this study is suitable as supporting data): No adverse effects were observed in Bluegill sunfish (*Lepomis macrochirus*) exposed to nominal concentrations of up to 10 x 0.27 µg a.s./L (spray) plus 5 x 0.87 µg a.s./L (slurry) from applications of up to 10 x 4.5 g a.s./ha (as spray) plus 5 x 7.2 g a.s./ha (as slurry) (≈ up to 10 x 0.8 g a.s./ha as spray plus 5 x 1.2 g a.s./ha as slurry to a 0.3 m deep water). Observations have been conducted for 4-5 months.

These studies conclusively demonstrate that the risk assessment based on laboratory data by far over-estimates the effect levels, which can be expected under field conditions.

Conclusion

Deltamethrin has a high acute toxicity to aquatic organisms when exposed under worst-case laboratory conditions. However, it was demonstrated in several higher tier studies that deltamethrin has a low toxicity or is non-toxic to fish under field conditions. Therefore, the use of a regulatory endpoint derived from laboratory data would represent a very conservative approach. **The RAC of 0.100 µg a.s./L based on the higher-tier microcosm study on Rainbow trout ([redacted], 2005; M-256604-01-1) is considered to be an adequate endpoint for the acute and chronic risk assessment for fish.**

Refined risk assessment for aquatic invertebrates

A high toxicity of deltamethrin to aquatic invertebrates, especially for the isopod *Asellus aquaticus*, was identified already during the last Annex I listing process. In the following, a refined risk assessment for aquatic invertebrates is presented taking into account new experimental studies and expert statements, which were performed after the last Annex I inclusion of deltamethrin, in addition to the already evaluated data.

Outdoor mesocosm studies

Two **higher tier outdoor mesocosm** studies investigating the effects of deltamethrin on aquatic invertebrates are available and summarized below ([redacted], 2001, M-200619-03-1 and [redacted] et al., 2005; M-246137-01-1). Of these studies only the [redacted] (2001) study was part of the Decis EC25 baseline dossier (KCP 10.2.2). Therefore, the results of both are compared here.

a) Outdoor mesocosm study with artificial exposure conditions ([redacted] 2001; already evaluated for last Annex I listing – M-200619-03-1; KCA 8.2.06)

To study the effects of deltamethrin under more realistic conditions than in standard laboratory studies, an outdoor mesocosm study on a natural freshwater community was conducted in 1 m³ enclosures. Deltamethrin EC 25 was applied under the water surface to static systems of 1 m depth and artificially mixed into the water body immediately thereafter. This stirring was a method required by the former Rapporteur Member State Sweden, but does not reflect the most realistic exposure conditions. Deltamethrin was applied three times at nominal concentrations of 1.0, 3.2, 10, 18, 32, 56, 100 and 180 ng a.s./L at 7-day intervals. Due to the expected sensitivity of *Asellus aquaticus*, this species was introduced into the mesocosms about two months before the first application to ensure its presence.

The results of this study show that 27 of 51 taxa determination groups, i.e. 53%, were neither directly nor indirectly affected by the test item, even at the highest test concentration of 180 ng a.s./L. They include species of Gastropoda, Hirudinea, Oligochaeta, sediment dwelling organisms, Insecta and several zooplankton organisms. The taxonomic richness of the aquatic ecosystem communities was not affected at concentrations up to and including 18 ng a.s./L. At higher levels, effects on taxonomic richness lasted for maximum 71 days, depending on the concentration and the organisms.

The effects found at the three lowest test levels, i.e. 1.0, 3.2 and 10 ng a.s./L, were similar. In total four groups of organisms were affected, the larval instars and emergent insects of the Chaoborids at all three levels, Ephemeroptera larvae at 3.2 and 10 ng a.s./L, the crustacean zooplankton species *Daphnia* spec. at 10 ng/L, and the Isopod species *Asellus aquaticus* at all three levels. However, the effects on Chaoborids, Ephemeroptera and *Daphnia* spec. were only short-term effects lasting between 8 and 13 days after the first application. The effects on *A. aquaticus* at 10 ng a.s./L lasted longer, i.e. for 65 days, until recovery was observed. However, it is difficult to judge whether or not the statistically significant effects detected at 1.0 and 3.2 ng a.s./L for *A. aquaticus* towards the end of the study (days of onset of effect: day 99 and day 71, respectively) were test item related. Such a delay in observable effects is rather unlikely for deltamethrin, which disappears rapidly from the water phase and is known for its fast knock-down effect.

All groups affected at ≤ 10 ng a.s./L recovered from the impact of the three test item applications between 21 and 85 days after the first treatment.

From the results of this mesocosm, it was concluded that the effects on the aquatic ecosystem observed after the application of 10 ng deltamethrin/L are acceptable as overall structure and function was not permanently affected. **A NOEAEC** ("no observed ecological adverse effect concentration") **of 3 x 10 ng a.s./L was derived.**

In the mesocosm study performed by [redacted] (2001, M-200619-03-1), the different concentrations of deltamethrin were mixed homogeneously into the water column immediately after application as requested by the former RMS Sweden. The mixing of the test item into the upper layer of the water column resulted in exaggerated exposure conditions, especially for organisms inhabiting deeper water layers or even the sediment surface. The exposure of aquatic invertebrates to deltamethrin in this study cannot be considered representative for spray applications of deltamethrin, as the test substance was not applied via spraying onto the water surface simulating drift, which is the only relevant entry route into water bodies for this insecticide. The results of this mesocosm study are therefore considered to show the toxicity of deltamethrin for this specific scenario rather than effects expected after spray application of deltamethrin.

It can be concluded, that the application method used by [redacted] (2001; M-200619-03-1) maximized the exposure conditions since it does not consider correctly

- the hydrophobic behaviour (surface spreading) of deltamethrin on the water surface,
- a possible dissipation (e.g. by indirect photolysis, volatilisation) of deltamethrin from the micro layer film on the water surface after spray drift,
- and the reduced exposure of sediment dwelling organisms when deltamethrin is applied via spray drift .

To simulate spray drift as the relevant entry route for deltamethrin under field conditions, another outdoor mesocosm study was performed by [redacted] (2005, M-246137-01-1) under more realistic exposure conditions:

b) Outdoor mesocosm study simulating the relevant route of entry ([redacted] 2005; M-246137-01-1)

A mesocosm study with Deltamethrin EW 15, the new representative formulation, was conducted simulating spray drift as the actual entry route. Outdoor tanks with 6 m^3 of water (1.0 m deep) and 15 cm natural sediment taken from an uncontaminated pond nearby were used for this study. The application rates targeted nominal concentrations of 4.8, 10.5, 23, 51 and 111 ng a.s./L (three applications at 7-day intervals). Spray drift was simulated by spraying the test item directly on the surface of the pond water. The study evaluated all immanent freshwater pelagic and benthic invertebrate species. Since most freshwater gammarids clearly prefer running water, this taxon was not tested in this static pond system but in bioassays performed in parallel to the mesocosm study ([redacted] 2005; M-246173-01-1).

As *G. aquaticus* can be considered representative for lentic water bodies of this type, this species was artificially introduced into the test ponds since a natural population was not available in the ponds. Because it was unclear whether the inserted populations could be maintained throughout the study, bioassays for this species were also performed with water and food samples taken from the

ponds throughout the study. The bioassays also allowed the assessment of potential recovery of an affected population.

Deltamethrin dissipated very rapidly from water by degradation and partitioning to sediment, with a mean DT_{50} value of 24 hours. Only two thirds of the total deltamethrin detected in pond water was dissolved in water, with the remaining third adsorbed to algae or particulate matter. The mean DT_{50} for the whole system (water plus sediment) was 32 hours.

Chaoborus crystallinus was identified as the most sensitive taxon with consistent effects even at 4.8 ng a.s./L. These occurred immediately after application until about a very few weeks after the last application when a full recovery was observed even at the highest test level. At 10.5 ng a.s./L, short-term effects were also observed for one Rotatoria species (*Keratella quadrata*) and Copepod nauplii. *A. aquaticus* showed a reduced activity at this test level for very few days after application without any sign of mortality or affected reproduction. At 23 and 56 ng a.s./L, effects on one to three more individual species were observed, but these effects were also short-term only, with full recovery being observed within the first weeks after the last application. The abundance of *Asellus* was clearly reduced after application at these concentrations but returned mostly to the level of controls until study termination. The differences between control and treatment levels were small and population abundance clearly increased in these ponds during the study, as additionally demonstrated by the increasing number of juvenile organisms. The bioassay findings confirmed that water and food samples taken from the mesocosms did not have any negative effects on *A. aquaticus* at the latest one week after the applications. At 111 ng a.s./L the number of affected zooplankton and insect species was distinctly higher, and the effects on *A. aquaticus* even more pronounced as compared to lower treatment levels.

The study author derived NOEAEC (“no observed ecological adverse effect concentration”) of 51 ng a.s./L, based on the observed effects on several invertebrate species at 51 ng a.s./L and the observed fast recovery of affected populations at 4.8 to 51 ng a.s./L. Because of the missing replication of the highest treatment level of 111 ng a.s./L, this concentration was not considered for the NOEAEC deduction.
Nevertheless, a NOEAEC of 23 ng a.s./L is suggested to be considered in this risk assessment as a more conservative approach, which is in line with the expert statement by [redacted] (2005; M-254687-01-1) that is provided below.
While a recovery of affected populations was clearly demonstrated for all pelagic or benthic invertebrate species at test concentrations up to and including 51 ng a.s./L, an in situ recovery of the *Asellus* population could not be demonstrated in this study at 23, 51 and 111 ng a.s./L. However, **taking into account the recovery potential of *Asellus aquaticus*, a NOEAEC of 23 ng a.s./L can be derived.**

An independent expert statement on the two mesocosm studies presented above is available and summarized in the following:

Evaluation report on higher-tier tests to assess the ecological risks of the insecticide deltamethrin to freshwater organisms ([redacted] 2005; M-254687-01-1)

In the context of this evaluation report, the author assessed the treatment-related effects observed in the mesocosm studies of [redacted] (2001; M-200619-03-1) and [redacted].

(2005; M-246137-01-1). Responses of the measurement endpoints were considered treatment-related when:

1. Clear concentration-response relationships were evident that could not be observed during the pre-treatment period
2. Statistically significant effects were demonstrated on at least two consecutive sampling dates
3. The statistically significant effects were ecologically relevant.

An overall summary of the "effect classes" determined by the author for several categories of endpoints is given in the tables below, for the detailed assessment reference is made to the original report. Within each category the most sensitive endpoint was selected. For the explanation of the effect classes reference is made to the summary provided under point CP10.2.3 of this document. In this evaluation report the author considers class 3 effects acceptable to derive the NOEAEC.

Summary table of study from Schanne & Van del Kolk, 2001:

Nominal peak concentration	1.0 ng/L	3.2 ng/L	10 ng/L	18 ng/L	32 ng/L	56 ng/L	100 ng/L	180 ng/L
Micro-Crustacea	1	1	3a	5a	5a	5b	5b	5b
Other zooplankters	1	1	1	1	3a *	3a *	3a *	3a *
Macro-Crustacea	2	2	3b	5b	5b	5b	5b	5b
Insects	2	3a	3a	3b	3b	3b	5b	5b
Other macro-invertebrates	1	1	1	1	1	3a *	3b *	3b *
Water quality endpoints	1	1	1	1	1	1	1	1

* = responses can at least in part be explained as resulting from indirect effects

The study of [redacted] (2001) revealed class 2 effects on two very sensitive taxa only at 1 ng deltamethrin/L, the lowest treatment-level tested (see table above). Since these effects were transient and slight, the overall **$NOEC_{community}$ of the study is set in this evaluation report at 1 ng deltamethrin/L**. When considering class 3a/3b effects (clear short-term effects) on a few populations of crustaceans (*Asellus*, *Daphnia*) and insects (*Chaoborus*, Ephemeroptera) acceptable **a NOEAEC of 10 ng deltamethrin can be derived from the study**. At treatment-levels of 18 ng deltamethrin/L and higher the results revealed clear long-term effects (class 5a/5b) on calanoid copepods and on *Asellus aquaticus*.

Summary for study of [redacted] 2005:

Nominal peak concentration	4.8 ng/L	10.5 ng/L	23 ng/L	51 ng/L	111 ng/L
Micro-Crustacea	1	3a	3a	3a	3a
Other zooplankters	2 *	2 *	3a *	3a *	3a *
Macro-Crustacea	1	2	3b - 5a? #	5a - 5b?	5b?
Insects	3a	3a	3a	3a	3b – 5b

Other macro-invertebrates	1	1	1	2 *	2 *
Phytoplankton	1	1	3a *	3a *	3a *
Water quality endpoints	1	1	1	1	1

* = responses can at least in part be explained as resulting from indirect effects

= The in situ bioassays show that potential recovery may be fast

An overall $NOEC_{community}$ cannot be derived from the experimental pond study provided by [redacted] (2005), since the lowest treatment-level (4.8 ng deltamethrin/L) resulted in class 3a effects on the phantom midge *Chaoborus*. At treatment-levels of 23 ng deltamethrin/L and higher, long-term effects on *Asellus aquaticus* cannot be excluded on basis of the responses observed in the microcosm study of [redacted] (2005). In situ bioassays performed with *Asellus aquaticus* and water from the outdoor microcosms, however, show that potential recovery of this isopod may be fast. For this reason **a NOEAEC of 23 ng deltamethrin/L can be derived when both the outdoor microcosm study and the in situ bioassays are considered**.

Taking into account the two mesocosm studies and related bioassays, the author comes to the following conclusions:

- The two outdoor semi-field tests reported by [redacted] (2001) and [redacted] (2005) can be used to evaluate the effects of short-term pulsed (3x, interval 7 d) deltamethrin exposure on freshwater communities.
- The study of [redacted] (2001) used test systems that had a relatively high diversity of freshwater arthropods. In this study relatively worst case exposure conditions were simulated, due to mixing of the test compound in the water column immediately after deltamethrin application.
- The study of [redacted] (2005) is characterised by test systems with a lower (but not exceptional for such model ecosystem studies) diversity of freshwater arthropods. However, several very sensitive arthropod populations (e.g. *Chaoborus*, *Asellus*) were present and additional bioassays with the sensitive macro-crustaceans *A. aquaticus* and *G. pulex* were performed. In addition, the study of [redacted] (2005) more realistically simulated the risks due to spray drift and described the stratification and dynamics in exposure concentrations in the course of the experiment in great detail.
- On basis of the most sensitive endpoints studied a $NOEC_{community}$ of approximately 1 ng deltamethrin/L can be derived from the study of [redacted] (2001).
- Under the assumption that short-term (class 3) effects on a few populations of sensitive arthropods are acceptable a NOEAEC of approximately 10 ng deltamethrin/L (based on nominal initial concentration) can be derived from the semi-field experiment reported by [redacted] (2001), and of 10 – 23 ng deltamethrin/L for the study reported by [redacted] (2005).
- Publications on the ecological effects of other pyrethroids in aquatic micro/mesocosms suggest that the NOEAEC of approximately 10 - 23 ng deltamethrin/L as observed in the studies reported by [redacted] (2001) and [redacted] (2005) can be used as an Environmentally Acceptable Concentration of deltamethrin in freshwater ecosystems (without

applying an extra Uncertainty Factor), at least if short term effects on a few insects and crustaceans are considered acceptable.

To address some open questions for the aquatic invertebrate risk assessment, which could not be fully answered by the mesocosm studies, additional studies and expert statements are presented in this document, which evaluate the risk to the following taxa in more detail:

1. *Chaoborus* (larvae) and other zooplankton species,
2. the amphipod *Gammarus*,
3. and the isopod species *Asellus aquaticus*

1. *Chaoborus* and other zooplankton species

Chaoborus was overall the most sensitive species within the mesocosm study ([redacted] 2005, M-246137-01-1), showing strong initial effects followed by a fast recovery of the affected population shortly after application. The following expert statement provides an analysis and interpretation of the **zooplankton dynamics** observed in the mesocosm ponds after the application of deltamethrin with special focus on the *Chaoborus crystallinus* population.

Analysis and interpretation of the zooplankton dynamics after application of Deltamethrin EW 015 to aquatic mesocosms with special focus on the *Chaoborus crystallinus* population
([redacted] 2007; M-291864-01-1)

Overall, ***Chaoborus crystallinus*** was found as the most sensitive species in the mesocosm study with deltamethrin demonstrating a distinct reduction in abundance of larvae and emerging midges immediately after the application at all treatment levels. However, larvae hatching from egg masses in the treated pond of the highest test level (111 ng a.s./L) already survived seven to eight days after the last application and emerged later on. The author concluded that the *Chaoborus crystallinus* population in the mesocosm probably recovered by means of external sources via egg masses laid on the water surface of treated ponds soon after the last application.

In addition the abundance of ***Daphnia longispina*** and **copepods** (mainly nauplii) was affected by deltamethrin at the highest test levels. Although the recovery for *D. longispina* was delayed by the predation of a growing population of *Chaoborus* larvae (see below), the populations of both, *Daphnia longispina* and copepods (mainly nauplii) recovered even up to the highest test level within some weeks after the last application at the latest. The population dynamics of *Chaoborus crystallinus* also caused some short-term indirect food web effects (as on rotifers and phytoplankton). Thus, the treatment with deltamethrin caused distinct short-term effects on a few zooplankton species, which also induced fluctuations on other zooplankton and phytoplankton species within the food web for some weeks only.

The results gained from the [redacted] (2005) mesocosm study allow for an interpretation of secondary effects caused by the population growth of a predator like *Chaoborus* or food competition between Cladocera/Copepoda and Rotatoria. These relationships cause delayed fluctuations and oscillating population dynamics for some time until a stable population density is reached

comparable to control findings. These food web interrelationships cannot be detected and evaluated in most mesocosm studies because of the natural fluctuations and variances between individual pond replicates. The mesocosm study discussed here, however, yields results that are precise enough to interpret and even to quantify such secondary effects as concluded by [redacted] (2007).

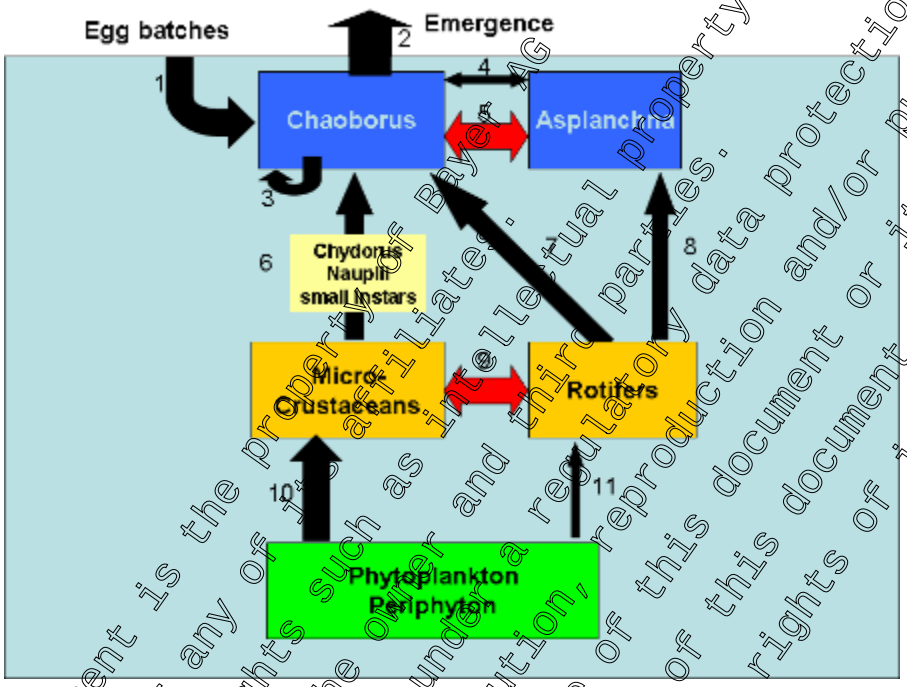


Figure 10.2- 1: Interrelationships between populations of plankton organisms as hypothesized for the pelagic compartment of a mesocosm (from: [redacted] 2007).
1) Entry of egg batches; 2) loss by emergence; 3) loss by cannibalism;
4, 6, 7, 8, 10, 11) losses by predation; 5, 9) competition via food sources

Following the food web evaluation it is seen as highly improbable that the test item had any direct effect on **rotifers**. On the contrary, the population growth of rotifers was promoted due to an indirect effect via the toxicant-induced short-term loss of effective predators (*Chaoborus*) and of competing *Cladocerans* (*Daphnia longispina, Chydorus sphaericus*), until the predators came into play again. *Asplanchna* and new young *chaoborid* larvae repopulating the mesocosms probably caused the sharp decline in rotifers soon after the applications. Thus, it can be assumed that all observed effects on the population dynamics of rotifers are to be considered as secondary effects of the treatment with deltamethrin.

Also, a dose-response relationship in population growth of ***Daphnia longispina*** was demonstrated. The abundance in the two highest test concentrations reached the range of the control group on day 63. Taking into consideration the rapid dissipation of the test item from the water phase and the short generation cycles (~10 d) of this species, the start of recovery appears rather delayed. Since the daphnid densities did not reach control densities until the first emergence of the adult chaoborids took place, it is highly probable that the growing population of 3rd- and 4th-instar *Chaoborus* larvae contributed by predation substantially to the delayed recovery. This is supported by the observations on the copepod populations, which was equally affected by the test item, but recovered more rapidly than the daphnid population, probably due to the fact, that the copepods are less frequently predated

by *Chaoborus* larvae, as they can easily escape from predation. It can be concluded that the delayed recovery of *Daphnia longispina* is a secondary effect rather than a direct result of deltamethrin exposure.

The secondary effects described are restricted to higher test concentrations, and the impacts on population densities are not very strong as compared to fluctuations of natural populations in control mesocosms. All observed direct and indirect effects recovered within some weeks after the application: the latest full recovery was observed for the rotifer *Keratella quadrata* and the cladoceran *Daphnia longispina* seven weeks after application. However, the generation time of these species is a few days only and, thus, the potential for the growth of an affected population is high. This period of seven weeks is shorter than a recovery period of eight weeks, which had been defined as an acceptability criterion for observed effects within the EU Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev. 4 (final) 2002).

2. *Gammarus* spec.

Based on acute laboratory endpoints for aquatic invertebrates listed in the US EPA "ECOTOX" database, [redacted] (2001; M-201584-01-1; KCA 8.2.08) identified the amphipod *Gammarus fasciatus* as the most sensitive species, which showed a higher sensitivity to deltamethrin than *Gammarus pulex*. However, *G. fasciatus* is a North American species mainly inhabiting upstream brooks with clear and fast flowing water, and is not considered relevant for European agricultural areas ([redacted], 2007; M-291865-01-1). Hence, this species was not selected as a test species for bioassays (see below), but *Gammarus pulex*, which is known to be sensitive to deltamethrin and other pyrethroids. This species is also the most common *Gammarus* species in streams in agricultural landscapes in Europe ([redacted], 2007).

Studies with *Gammarus fasciatus*, conducted with the former representative formulation Deltamethrin EC 25 were evaluated for the last Annex I listing of deltamethrin.

Test species	Test substance	Test system	Endpoint	Reference
Gammarus fasciatus	Deltamethrin EC25	96 h, flow-through, water only	LC_{50} = 0.31 ng a.s./L (mm) LC_{50} = 3.2 ng a.s./L (nom)	[redacted] (2000) M-194285-01-1 KCA 8.2.4/03
Gammarus fasciatus	Deltamethrin EC25	96 h, single pulse exposure, water-sediment system	LC_{50} > 43 ng a.s./L (nom)	[redacted] (2000) M-198400-01-1 KCA 8.2.4/04

mm = mean measured, nom = nominal

The results demonstrate that the toxicity to this species is considerably reduced under more realistic exposure conditions.

In addition, new **bioassays with the amphipod *Gammarus pulex*** were performed in parallel to the mesocosm study of [redacted] (2005) ([redacted], 2005; M-246173-01-1) with water and leave samples taken from the mesocosm ponds at different sampling dates after the deltamethrin applications. Leaves function both as habitat and food for *Gammarus* species. **A NOEC of 23 ng a.s./L was derived from this study for *Gammarus pulex*** based on nominal peak concentrations. The

bioassays showed mortality of test organisms only in samples taken at higher concentrations shortly after the deltamethrin applications, demonstrating a full recovery potential of affected populations under field conditions for all test concentrations (up to 111 ng a.s./L) already after about one week after application of the test item.

Gammarus pulex was less sensitive in these bioassays than *Asellus aquaticus* (NOEC = 10.5 ng a.s./L, see below), confirming the results from Tier 1 laboratory studies (see [redacted] 2001; M-201581-01-1, KCA 8.2/08). Therefore, unacceptable effects on relevant *Gammarus* species are not expected at environmental concentrations of deltamethrin, which are considered safe for *A. aquaticus*.

3. *Asellus aquaticus*

The isopod *Asellus aquaticus* was identified as another sensitive species to deltamethrin in laboratory studies (see [redacted] 2001; M-201581-01-1; KCA 8.2/08) and in the mesocosm study of [redacted] (2001; M-200619-03-1; KCA 8.2/06). Therefore, it was also artificially introduced into the ponds of the mesocosm study of [redacted] (2005; see M-246137-01-1) and selected as test species for the corresponding bioassays. The study is summarized under point CP 10.2.3 of this document, and the general results were discussed above. Nevertheless, the effects observed for the isopod *Asellus aquaticus* are presented here in further detail:

Effects on *A. aquaticus* in the outdoor mesocosm study with Deltamethrin EW15 ([redacted] 2005; M-246137-01-1)

When interpreting the *Asellus* results of the mesocosm studies, one has to bear in mind, that both sampling devices used for *A. aquaticus*, i.e. artificial substrate samplers (ASS) and leaf cages, are mainly activity measures of this species ("activity traps"). The efficiency of these methods is influenced by other competing factors such as availability of food in the mesocosm ponds (e.g. macrophytes). Thus, reduced numbers of trapped individuals do not necessarily reflect mortality, and results from these samplings do not allow for a conclusive interpretation of population dynamics.

Three applications of 4.8 and 10.5 ng a.s./L deltamethrin at a 7-day interval did not cause relevant effects on the activity or mortalities of exposed adult and juvenile organisms, **resulting in a NOEC for the mesocosm and and bioassay of 10.5 ng a.s./L for *Asellus aquaticus*.**

Table 10.2-4: No-Observed-Effect-Concentrations (in ng a.s./L) of *Asellus aquaticus* in the mesocosms as obtained from statistical testing

Day(s)	Leaf cages	Artificial substrate samplers (ASS)	Leaf cages & ASS
-1 - 0 (pre-application)	>111	>111	>111
2	4.8#	>111	10.5
4	>111	>111	4.8#
7	23.0	>111	10.5
9	>111	>111	>111
11	>111	>111	>111
14	10.5	>111	>111
16	4.8#	10.5	4.8#
18	10.5	10.5	10.5
21	>111	>111	>111

29 - 105	≥10.5*	≥10.5*	≥10.5*
Consistent NOEC	**10.5**	**10.5**	**10.5**

* Test concentrations above 10.5 ng a.s./L could not be derived as NOEC after day 21, because one of the two replicates was used to simulate immigration at higher concentrations.

A NOEC of 4.8 ng a.s./L was determined for single sampling dates only. A consistent NOEC determination for a mesocosm study, should be based on at least two consecutive sampling dates

At 10.5 ng a.s./L a slight reduction in the activity of adult and juvenile *A. aquaticus* was observed for very few days after the first application only, most likely due to the well-known effect of pyrethroids to cause short-term paralysis of invertebrates at low exposure concentrations. However, the later findings clearly indicate no mortality at this test concentration, since the abundance of "trapped" individuals was the same as in control ponds at the following sampling days and was not reduced after the second and third application (see **Figure 10.2- 2**).

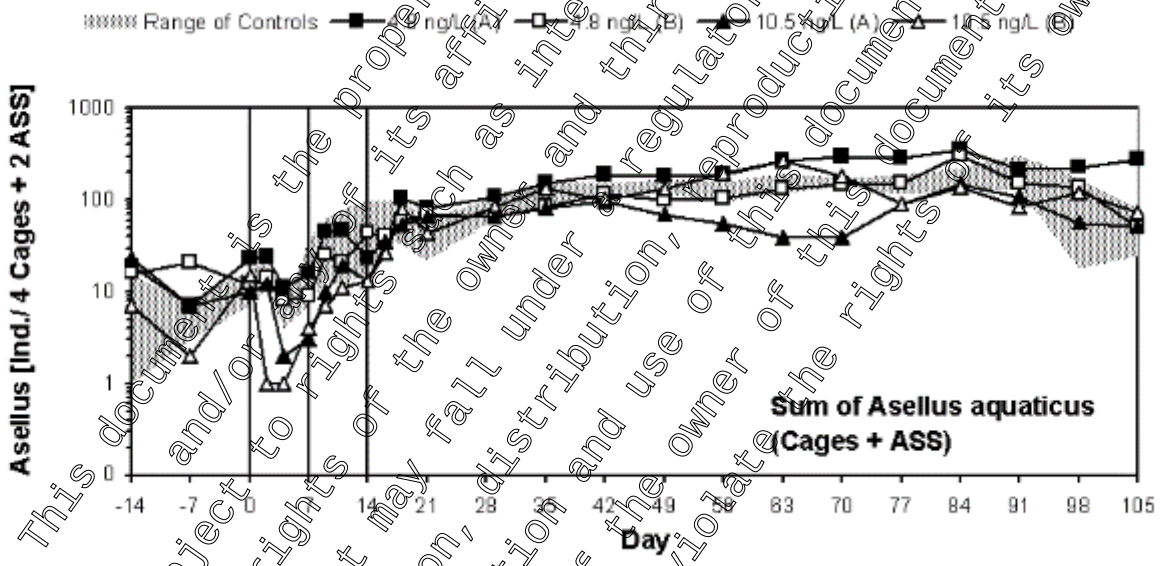


Figure 10.2- 2: Sum of *Asellus aquaticus* in the leaf cages and ASS following application of deltamethrin at nominal concentrations of 4.8 and 10.5 ng a.s./L, respectively. The vertical lines indicate the applications.

In the three highest test concentrations of 23, 51 and 111 ng a.s./L the mobility of *A. aquaticus* during the treatment period was clearly reduced in all ponds. However, one to two weeks after the third application the number of mobile (i.e. trapped) individuals clearly increased in one pond of each of the 23 and 51 ng a.s./L treatments and the abundance reached the control level by the end of the study at the latest in both treatment groups (see **Figure 10.2- 3**).

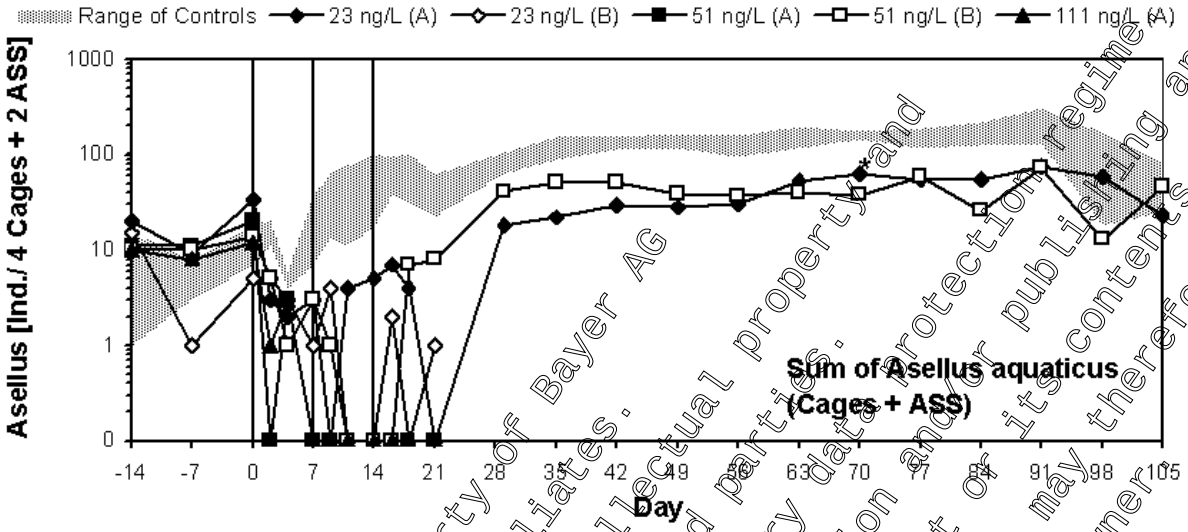


Figure 10.2- 3: Sum of *Asellus aquaticus* in the leaf cages and ASS following application of deltamethrin at nominal concentrations of 23 and 51 ng/L, respectively. The vertical lines indicate the applications.
In the replicates 23 ng/L (B) and 51 ng/L (A), additional *Asellus* from the culture were inserted at day 21 to simulate immigration. Therefore, these replicates are considered in the graph above only until day 21.
* On day 63, 124 *Asellus* individuals from control B were inserted into pond 23 ng/L (A) by mistake.

The number of mobile *A. aquaticus* was low seven days after the third application in all ponds of the three highest test concentrations. Hence, the study performers decided to introduce further individuals of *A. aquaticus* from the culture to one replicate of each of these test concentrations as well as to the highest test concentration of 111 ng a.s./L in order to simulate immigration.

At 23 ng a.s./L the numbers of mobile *Asellus* slowly increased after the introduction of new individuals in these additionally inoculated ponds and reached the same abundance as in control ponds eight weeks after the last application – although control ponds can no longer be considered as fully valid controls for these ponds. At the two highest test concentrations, the number of sampled mobile *Asellus* fluctuated and remained nearly constant for the rest of the study. At the end of the study the abundance even at 111 ng a.s./L was clearly within the range of the control.

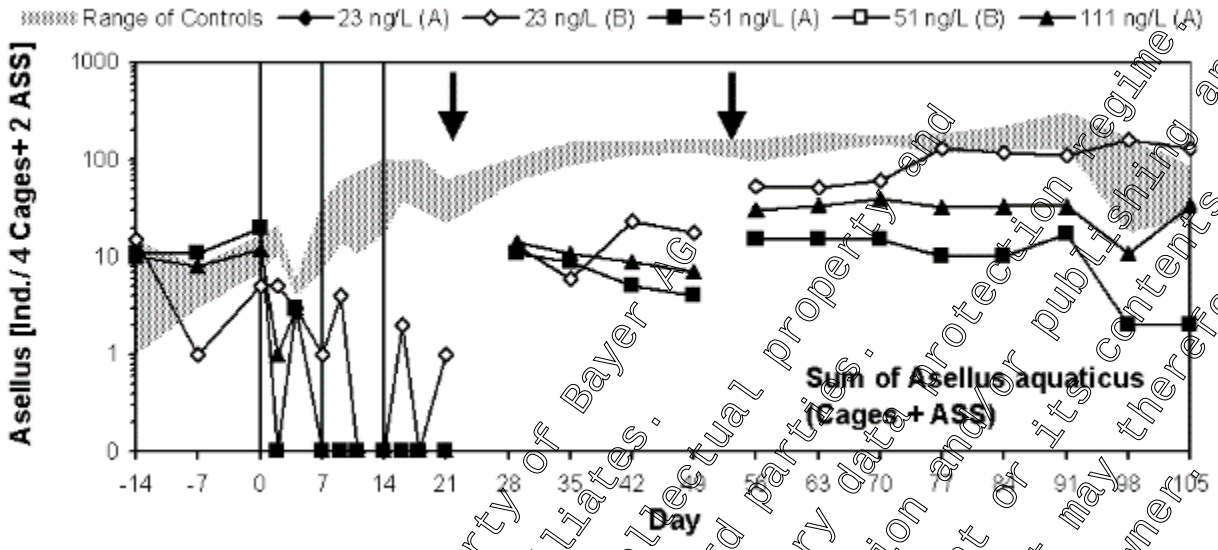


Figure 10.2- 4: Sum of *Asellus aquaticus* in the leaf cages and ASS following application of deltamethrin in those replicates of the three highest test concentrations, to which additional *Asellus* individuals were inserted to simulate immigration. The vertical lines indicate the applications. The arrows indicate the additional insertion of *Asellus* from the culture.

After day 70, the proportion of juveniles at these test levels also reached the level of control ponds. However, a full recovery to the control level within eight weeks after the last application could not be demonstrated without doubt for 23, 51 and 111 ng a.s./L. Nevertheless, the differences between control and treatment levels are small and population abundances clearly increased in these ponds, as also demonstrated by the increasing number of juvenile organisms and the corresponding reproduction in situ. Since *Asellus* has a long generation time of several weeks under the conditions of this study, it cannot be expected that this species could have built up the same population density as in control ponds within a few weeks only. The bioassays performed in parallel showed that three weeks after the first application (one week after last application) survival of immigrating *Asellus* would no longer be affected by treated pond water and exposed leaves even at the highest test concentration, demonstrating the recovery potential of an impacted *A. aquaticus* population.
However, since control ponds cannot be used for a direct comparison after the insertion of further *Asellus* individuals, these additionally inoculated mesocosm replicates have to be interpreted with care and should be used as supporting information only.

The corresponding **bioassays** conducted with mesocosm water and leave material from different sampling dates showed effects on survival of adults at test concentrations ≥ 23 ng a.s./L only shortly after the applications, indicating a recovery potential for all test concentrations including the highest one (111 ng a.s./L) as early as about one week after application.

Overall, the findings suggest, that effects on *Asellus aquaticus* observed at concentrations up 23 ng a.s./L can be considered as acceptable, based on the assumption that recolonisation will occur in natural water bodies of the agricultural landscape.

*****

Effects on *A. aquaticus* in an outdoor mesocosm study with the formulation Thiacloprid & Deltamethrin OD 100+10 ([redacted] 2005; M-259938-01-2)

An additional outdoor mesocosm study is available for the formulation Thiacloprid & Deltamethrin OD 100+10, with a test design comparable to the deltamethrin mesocosm ([redacted], 2005, M-246137-01-1, see above): the sensitive isopod *Asellus aquaticus* was introduced to the mesocosms, immigration was simulated at the higher concentrations and a bioassay with *Asellus* was performed in parallel. The mesocosms were exposed to a single application of the product, simulating spray drift. The treatment levels were 0.5, 1.1, 2.5, 5.5, and 11.9 µg test item/L, corresponding to 50.4, 110, 242, 536, and 1166 ng thiacloprid/L and 4.8, 10.5, 23, 51 and 111 ng deltamethrin/L, respectively.
Effects were observed at all treatment levels: at the lowest level (0.5 µg prod./L, equivalent to 4.8 ng deltamethrin/L), a slight reduction of activity was observed, but population growth was possible again within a few weeks. Due to unlimited growth of the control population, the control levels could not be reached, however, the population growth rate was comparable. At higher concentrations, populations became extinct or almost extinct. However, up to 5.5 µg prod./L (equivalent to 51 ng deltamethrin/L) possible recolonisation after two weeks was demonstrated by insertion of new organisms into test ponds. At the highest treatment level, survival of immigrants was possible at least after seven weeks. Laboratory bioassays also showed after eight weeks full survival up to the highest treatment level.
A NOEAEC of 2.5 µg product/L was derived by the study director, which is equivalent to a deltamethrin concentration of 23 ng a.s./L. Although the results of this mesocosm study are considered supporting information only - as it covers only a single application, and the second active substance of the formulation (the insecticide thiacloprid) may have contributed to the observed effects - the outcome of this study supports the overall conclusion, that **effects of deltamethrin on aquatic invertebrate species, especially the isopod *A. aquaticus*, are observed only for a short period of time followed by recovery at application rates up to 23 ng deltamethrin/L.**

Small, fully treated pond mesocosms represent a worst-case situation for recovery of species like *Asellus*, which do not have resting stages (like e.g. daphnids) and are not able to immigrate by flying life stages. However, the successful artificial immigration and the survival in the bioassays demonstrate the recovery potential in the field by immigration of organisms from adjacent water bodies or unaffected areas of the same water body. To examine the actual mobility, and hence – recolonisation potential - of *Asellus aquaticus,* a field experiment was conducted in a representative water body of the agricultural landscape:

Field experiments on the drifting behavior of Asellus aquaticus in an agricultural stream ([redacted] 2007, M-291925-01-1).

Drifting is a special behavioural response of lotic animals to specific environmental conditions. To gain some information on the drift behaviour of *Asellus aquaticus* experiments were performed in an agricultural stream (Birnbach) close to Landau, Southwest Germany. The Birnbach is a third order stream discharging agricultural land (mainly vineyards). It has a permanent discharge of about 10 L/s at a depth between 10 and 20 cm. The streambed is between 50 and 150 cm wide and has a sandy-loamy substrate, which is covered by macrophytes and filamentous green algae (coverage: 50% to 60%). The

flow velocity during the observation time between July and August was between 0.01 and 0.31 m/s with an average of 0.05±0.06 m/s (n=68). In consistence with the EU FOCUS scenarios, the Birnbach is comparable with a slow flowing ditch. The average density of *Asellus* found in the Birnbach was relatively high (average of 2223±1040 individuals/m^2) (n=15) and was comparable to densities reported in literature from different countries (Iversen & Thorup, 1998[1]; Graca et al. 1994[2] and Petridis 1990[3]).
Experiments were performed to observe the total abundance of *Asellus* in this stream and their drift behavior (drift rate and drift distance). Sampling with drift nets over 24 hours resulted in **drift rates** between 225 and 1918 individuals/24 h (mean drift rate: 675 ± 467 individuals/24 h, n=45). Regarding the **drift distance**, the observed drift pattern suggested that distances of 14 m and 25 m were of relevance for *A. aquaticus* in the two experiments conducted.
Based on the results the authors concluded: "Even if it is not conclusively possible to distinguish between active or passive components or between drift and locomotion, the data from the study reported here still **suggest a rather high spatial dynamic for the isopod species *A. aquaticus*."**

These results support the assumption, that recovery of an *Asellus* population in natural water bodies in the field, e.g. ditches, occurs faster than possible in the isolated mesocosm ponds.

To receive additional information on the toxicity of deltamethrin to *Asellus aquaticus* a study was performed with different life stages of the isopod *A. aquaticus* L. (Isopoda) under realistic spray exposure conditions in the laboratory:

Acute and chronic effects of deltamethrin to different life stages of *Asellus aquaticus* ([redacted] 2007; M-291885-02-1 and M-291879-01-1)

In the first study, conducted with the new representative formulation Deltamethrin EW 15, the test organisms were exposed to nominal concentrations of 1.0, 2.2, 4.8, 10.6, 23.4 and 51.5 ng a.s./L (overspray application, simulating spray drift) in a natural water-sediment system for a period of 21 days. The observed toxicity of deltamethrin to *A. aquaticus* after 24 hours in this study was in the same range as after 48 hours and up to 21 days after application. Since it was not possible to find all introduced individuals at the interim sampling dates due to technical reasons, the final evaluation on day 21 is considered the most relevant for the risk assessment. After 21 days LC_{50} values were determined to be 43.9 ng a.s./L for adult and 44.8 ng a.s./L for juvenile *A. aquaticus*, respectively, based on nominal concentrations. These results indicate that the sensitivity of juvenile *A. aquaticus* to deltamethrin was the same as for adults.
In the vessels where adults had been introduced, newborn *A. aquaticus* were already observed four days after application up to a concentration of 23.4 ng a.s./L. At 51.5 ng a.s /L newborns were only observed 14 days after application.
Based on mortality the 21-day NOEC for adult and juvenile *Asellus aquaticus* was determined to be 23.4 ng a.s./L.

[1] Iversen, T. M. & Thorup, J. (1988): A three years' study of life cycle, population dynamics and production of *Asellus aquaticus* L. in a macrophyte rich stream. Internationale Revue der gesamten Hydrobiologie 73:73-94.
[2] Graca, M.A.S.; Maltby, L. & Calow, P. (1994): Comparative ecology of *Gammarus pulex* (L.) and *Asellus aquaticus* (L.) I: population dynamics and microdistribution. - Hydrobiologia 281:155-162.
[3] Petridis D. (1990): Influence of grass carp and tench on the ecology of *Asellus aquaticus*. Archiv für Hydrobiologie 118:105-124.

The findings of this life stage study are well confirmed by the results of a second, laboratory population study with *Asellus aquaticus* ([redacted], 2007; M-291879-01-1). The study was aimed to determine the chronic effects (such as population dynamics and potential recovery) of deltamethrin towards a population of different age (size) classes of *Asellus aquaticus* in a water-sediment system under realistic spray exposure conditions. However, since the life stage study provided reliable results and the interpretation of results from a population study with different age classes is difficult, the population study was terminated already five weeks after study implementation. Nevertheless, the results obtained confirm the results of the life stage study as discussed above: **survival of juvenile and adult *A. aquaticus* was not affected after 35 days of exposure to deltamethrin in a static water-sediment-system up to a nominal peak concentration of 51.5 ng a.s./L**. No difference in sensitivity between juvenile and adult organisms was observed.

The results of these laboratory studies on *Asellus aquaticus* are in agreement with those from the [redacted]. mesocosm study. Since deltamethrin had been sprayed three times at a seven-day interval in the mesocosm study the biological effects are slightly more pronounced as compared to the laboratory studies with a single application only. The proposed use pattern according to the GAP is a single application in sugarbeet, and two applications at a 14-day interval in cauliflower and wheat, respectively. Hence, the three applications at a 7-day interval in the mesocosm from [redacted] (2005) can be considered a conservative scenario.

Overall summary of the presented higher tier studies and derivation of an endpoint for the aquatic invertebrate risk assessment

The available data indicate a high acute sensitivity of several invertebrate species (mainly insects and macro-crustaceans) to deltamethrin in acute laboratory studies under flow-through or semi-static conditions in water only studies. Because of the low water solubility of the substance and its high adsorption to organic material, the toxicity is distinctly reduced under realistic environmental conditions. Therefore, a number of additional higher tier studies were conducted as described above. An overview of the respective endpoints is given in the table below:

Table 10.2- 5: Overview of higher tier studies conducted with deltamethrin to support the risk assessment for aquatic invertebrates

Test species	Test substance	Test system/Activity	Results/Endpoint	Reference
Mesocosm studies				
Freshwater community	Deltamethrin EC25	**Outdoor mesocosm** 3 appl. mixed into water, appl. interval 7 d	NOEAEC 3 x 10 ng a.s./L	[redacted] (2001) M-200619-03-1 KCA 8.2.7/06
Freshwater community	Deltamethrin EW15	**Outdoor mesocosm** 3 appl. sprayed onto water surface, appl. interval 7 d	NOEAEC 3 x 23 ng a.s./L#	[redacted] (2005) KCP 10.2.3/02 M-246137-01-1
Freshwater community	Thiacloprid + Deltamethrin OD 100+10	**Outdoor mesocosm** Single spray application onto water surface	NOEAEC 23 ng a.s./L*	[redacted] (2005) KCP 10.2.3/06 M-259938-01-2
Scientific evaluations				
Freshwater community	*Deltamethrin*	Analysis of the mesocosm data of [redacted] (2001) and [redacted] et al. (2005), incl. bioassays for *Asellus*	NOEAEC 10-23 ng a.s./L	[redacted] (2005) KCP 10.2.3/04 M-254687-01-1
Zooplankton (incl. *Chaoborus crystallinus*)	Deltamethrin EW15	Analysis of zooplankton dynamics in [redacted] (2005) mesocosm with special focus on *C. crystallinus*	NOEAEC 111 ng a.s./L	[redacted] (2007) KCP 10.2.3/05, M-291864-01-1
Gammarus				
Gammarus pulex	Deltamethrin EW15	**Bioassay** with water from mesocosm study to assess potential for recovery	NOEC 23 ng a.s./L	[redacted] & [redacted] (2005) KCP 10.2.3/03 M-246173-01-1
Gammarus fasciatus	Deltamethrin EC25	Lab study, 96 h, flow-through, water only	LC_{50} 0.31 ng a.s./L (mm) LC_{50} 3.2 ng a.s./L (nom)	[redacted] (2000) M-194285-01-1 KCA 8.2.4/03
Gammarus fasciatus	Deltamethrin EC25	Lab study, 96 h, single pulse exposure, water-sediment system	LC_{50} >43 ng a.s./L (nom)	[redacted] (2000) M-198400-01-1 Decis EC25 baseline dossier KCP 10.2.1
Asellus				
Asellus aquaticus	Deltamethrin EW15	**Bioassay** with water from mesocosm study to assess potential for recovery	NOEC 10.5 ng a.s./L	[redacted] (2005) KCP 10.2.3/02 M-246137-01-1
Asellus aquaticus	Deltamethrin EW15	Lab study with different life stages of *A. aquaticus*, single application to static water sediment system, 21 d	NOEC 23.4 ng a.s./L $LC_{50\ adult}$ 43.9 ng a.s./L $LC_{50\ juvenile}$ 44.8 ng a.s./L	[redacted] (2007) KCP 10.2.3/07 M-291885-02-1

Asellus aquaticus	n.a.	Generic field study to examine drift behavior of *A. aquaticus*	Results suggest a rather high spatial dynamic for the species.	[redacted] (2007) KCP 10.2.3/12 M-299925-01-1

[#] A NOEAEC of 3 x 51 ng a.s./L is derived by [redacted] et al. (2005) in the original report. However, in a conservative approach, the NOEAEC of 3 x 23 ng a.s./L is considered in the risk assessment provided in this document.

[*] Endpoint recalculated for the active substance deltamethrin based on its content of 0.938% w/w in the test item Thiacloprid + Deltamethrin OD 100+10. As thiacloprid may have contributed to the observed effects, this endpoint is of limited reliability and considered as supporting information only.

The mesocosm study of [redacted]. (2005) confirms the high sensitivity of insects and macro-crustaceans to deltamethrin but shows a reduced toxicity compared to the results of laboratory studies under artificial test conditions. It also demonstrates that the observed effects on various taxa (e.g. *Chaoborus*) are of short duration only, with a fast recovery of all affected populations within a few weeks (except for *A. aquaticus*, see argumentation below). Based on the demonstrated fast recovery of affected populations at 4.8 to 23 ng a.s./L, a NOEAEC ("no observed ecological adverse effect concentration") of 23 ng a.s./L can be derived from this mesocosm study. It has to be noted, that this mesocosm covered three applications at a 7-day interval, whereas the GAP proposes a maximum of two applications at a 14-day interval. Therefore the results from this study can be considered a conservative scenario.

The bioassays also confirm that *Asellus aquaticus* is more sensitive than *Gammarus pulex* (NOEC = 23 ng a.s./L), the relevant Gammarid species for agricultural landscapes in Europe. A risk assessment based on the sensitive species *A. aquaticus*, is therefore expected to cover also the risk to gammarids.

The demonstration of an *in situ* recovery of the *Asellus* population (NOEC = 10 ng a.s./L) could not be demonstrated in the [redacted]. (2005) mesocosm at concentrations ≥ 23 ng a.s./L. However, the complementary bioassays on *A. aquaticus* demonstrated the potential for recovery within a few days after application. This is supported by the increasing numbers of juvenile organisms detected during the end of the study. Moreover, the sampling devices used for *A. aquaticus* are primarily "activity traps". Reduced numbers of trapped individuals do not necessarily reflect mortality, and results from these samplings do not allow for a conclusive interpretation of population dynamics. The missing recovery of *Asellus* in the mesocosms is most likely due to the isolation of the individual test ponds, and hence, a missing recolonisation potential of this species during the study. A field study on the drift behaviour of *A. aquaticus* showed a rather high mobility of this species, supporting the conclusion that recovery of *Asellus* via recolonisation can be expected in natural water bodies in the agricultural landscape.

In addition, acute and chronic effects of a single application of deltamethrin to different life stages of *Asellus aquaticus* were investigated in a water-sediment laboratory study ([redacted], 2007). The results of this study show that no life-stage depended sensitivity to deltamethrin exposure could be observed. The NOEC derived from this study was 23.4 ng a.s./L for both life stages. A population study performed in parallel confirmed the outcome of the life stage study.

Taking into account this additional information on *Asellus aquaticus*, and its recovery potential under field conditions, a NOEAEC of 23 ng a.s./L can be derived for this species. This conclusion is also supported by an independent expert statement of [redacted] (2005; M-254687-01-1).

Taking into consideration all data available, an overall NOEAEC$_{community}$ of 23 ng a.s./L is proposed for deltamethrin. No unacceptable effects on aquatic invertebrates are expected at this surface water concentration.

******

Predicted environmental concentrations used in risk assessment

Predicted environmental concentrations of deltamethrin in surface water were calculated according to FOCUS for the uses of Deltamethrin EW15 in sugarbeet, cauliflower, spring and winter wheat. PECsw values that are relevant for the aquatic risk assessment are summarized in the following tables. For details of PEC calculations refer to MCP Sec. 9 Point 9.2.5.

Table 10.2- 6: Maximum PECsw values – FOCUS Step 1 & 2

Compound	FOCUS Scenario	Sugarbeet 1 x 7.5 g a.s./ha	Cauliflower 2 x 7.5 g a.s./ha	Spring wheat 2 x 6.25 g a.s./ha	Winter wheat 2 x 6.25 g a.s./ha
		$PEC_{sw, max}$ [µg/L]	**$PEC_{sw, max}$ [µg/L]**	**$PEC_{sw, max}$ [µg/L]**	**$PEC_{sw, max}$ [µg/L]**
Deltamethrin	STEP 1	0.0692	0.1383	0.1153	0.1153
	STEP 2 - North	**0.0690**	**0.0690**	**0.0575**	**0.0575**
	STEP 2 - South	**0.0690**	**0.0690**	**0.0575**	**0.0575**
4'OH-deltamethrin (BCS-BY84407)	STEP 1	0.0007	0.0015	0.0012	0.0012
	STEP 2 - North	**0.0007**	**0.0013**	0.0010	**0.0010**
	STEP 2 - South	**0.0007**	**0.0013**	**0.0011**	**0.0010**
Br_2CA (AE F108565)	STEP 1	0.3411	0.6823	0.5685	0.5685
	STEP 2 - North	0.0400	0.0446	0.0371	0.0282
	STEP 2 - South	**0.0702**	**0.0769**	**0.0641**	**0.0461**
Serinyl-BrCA (BCS-CW57835)	STEP 1	0.0033	0.0066	0.0055	0.0055
	STEP 2 - North	**0.0033**	**0.0057**	**0.0048**	**0.0048**
	STEP 2 - South	**0.0033**	**0.0057**	**0.0048**	**0.0048**
mPBacid (AE F109036)	STEP 1	0.0522	0.1045	0.0871	0.0871
	STEP 2 - North	**0.0032**	**0.0053**	**0.0044**	**0.0044**
	STEP 2 - South	**0.0032**	**0.0053**	**0.0044**	**0.0044**

For Step 2, the worst-case value, resulting either from single of multiple application is given, for crops where more than one application is intended.
Bold values are considered in the risk assessment.

Table 10.2- 7: Maximum PEC_{sw} values – FOCUS Step 3

Compound	FOCUS Scenario	Sugarbeet	Cauliflower	Spring wheat	Winter wheat
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Deltamethrin	D1 (ditch)	-	-	0.0401	0.0400
	D1 (stream)	-	-	0.0316	0.0307
	D2 (ditch)	-		-	0.0402
	D2 (stream)	-	-	-	0.0332
	D3 (ditch)	0.0393	0.0476	0.0399	0.0399
	D4 (pond)	0.0016	0.0017	0.0014	0.0014
	D4 (stream)	0.0325	0.0378	0.0321	0.0317
	D5 (pond)	-	-	0.0014	0.0014
	D5 (stream)	-	-	0.0313	0.0321
	D6 (ditch)	-	0.0464	-	0.0402
	R1 (pond)	0.0016	0.0017	-	0.0014
	R1 (stream)	0.0272	0.0315	-	0.0263
	R2 (stream)	-	0.0422	-	-
	R3 (stream)	0.0382	0.0442	-	0.0369
	R4 (stream)	-	0.0313	0.0263	0.0263

The worst-case value, resulting either from single or multiple application is given, for crops where more than one application is intended.
Maximum PECsw values including the amount of deltamethrin sorbed to suspended solids are considered in the aquatic risk assessment as a worst-case approach.

Table 10.2- 8: Maximum PEC_{sw} values for deltamethrin – FOCUS Step 4

Compound	FOCUS Scenario	Sugarbeet 1 x 7.5 g a.s./ha	Cauliflower 2 x 7.5 g a.s./ha	Spring wheat 2 x 6.25 g a.s./ha	Winter wheat 2 x 6.25 g a.s./ha
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
5 m buffer zone					
Deltamethrin	D1 (ditch)	-	-	0.0109	0.0109
	D1 (stream)	-	-	0.0114	0.0111
	D2 (ditch)	-	-	-	0.0109
	D2 (stream)	-	-	-	0.0120
	D3 (ditch)	0.0128	0.0128	0.0109	0.0109
	D4 (pond)	0.0014	0.0014	0.0012	0.0012
	D4 (stream)	0.0137	0.0138	0.0116	0.0114
	D5 (pond)	-	-	0.0012	0.0012
	D5 (stream)	-	-	0.0113	0.0116
	D6 (ditch)	-	0.0125	-	0.0109
	R1 (pond)	0.0014	0.0014	-	0.0012
	R1 (stream)	0.0114	0.0115	-	0.0095
	R2 (stream)	-	0.0154	-	-
	R3 (stream)	0.0161	0.0161	-	0.0133
	R4 (stream)	-	0.0114	0.0095	0.0095

The worst-case value, resulting either from single or multiple application is given, for crops where more than one application is intended.
Maximum PECsw values including the amount of deltamethrin sorbed to suspended solids are considered in the aquatic risk assessment as a worst-case approach.

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 9: TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Sugarbeet					
Deltamethrin	Fish, acute	LC_{50} 0.15	0.0690	**2.2**	100
	Invertebrate, acute (*D.magna*)	EC_{50} 0.0131		**0.2**	100
	Invertebrate, acute (*A. bahia*)	LC_{50} 0.0037		**0.05**	100
	Invertebrate, acute (*H. azteca*)	EC_{50} 0.00017		**0.002**	100
4'OH-deltamethrin (BCS-BY84407)	Fish, acute	LC_{50} 3.99	0.0007	5700	100
	Invertebrate, acute	EC_{50} 670		957143	100
Br_2CA (AE F108565)	Fish, acute	LC_{50} 100000	0.0702	>1424501	100
	Invertebrate, acute	EC_{50} >100000		>1424501	100
Serinyl-BrCA (BCS-CW57835)	Invertebrate, acute	EC_{50} 35300	0.0033	10696969	100
mPBacid (AE F109036)	Fish, acute	LC_{50} 13300	0.0032	4156250	100
	Invertebrate, acute	EC_{50} 85000		26562500	100
Cauliflower					
Deltamethrin	Fish, acute	LC_{50} 0.15	0.0690	**2.2**	100
	Invertebrate, acute (*D.magna*)	EC_{50} 0.0131		**0.2**	100
	Invertebrate, acute (*A. bahia*)	LC_{50} 0.0037		**0.05**	100
	Invertebrate, acute (*H. azteca*)	EC_{50} 0.00017		**0.002**	100
4'OH-deltamethrin (BCS-BY84407)	Fish, acute	LC_{50} 3.99	0.0013	3069	100
	Invertebrate, acute	EC_{50} 670		515385	100
Br_2CA (AE F108565)	Fish, acute	LC_{50} 100000	0.0769	1300390	100
	Invertebrate, acute	EC_{50} >100000		>1300390	100
Serinyl-BrCA (BCS-CW57835)	Invertebrate, acute	EC_{50} 35300	0.0057	6192982	100
mPBacid (AE F109036)	Fish, acute	LC_{50} 13300	0.0053	2509434	100
	Invertebrate, acute	EC_{50} 85000		16037735	100
Spring wheat					
Deltamethrin	Fish, acute	LC_{50} 0.15	0.0575	**2.6**	100
	Invertebrate, acute (*D.magna*)	EC_{50} 0.0131		**0.2**	100
	Invertebrate, acute (*A. bahia*)	LC_{50} 0.0037		**0.06**	100
	Invertebrate, acute (*H. azteca*)	EC_{50} 0.00017		**0.003**	100
4'OH-deltamethrin (BCS-BY84407)	Fish, acute	LC_{50} 3.99	0.0011	3627	100
	Invertebrate, acute	EC_{50} 670		609091	100
Br_2CA (AE F108565)	Fish, acute	LC_{50} 100000	0.0641	1560062	100
	Invertebrate, acute	EC_{50} >100000		>1560062	100

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Serinyl-BrCA (BCS-CW57835)	Invertebrate, acute	EC_{50}	35300	0.0048	7354167	100
mPBacid (AE F109036)	Fish, acute	LC_{50}	13300	0.0044	3022727	100
	Invertebrate, acute	EC_{50}	85000		19318181	100
Winter wheat						
Deltamethrin	Fish, acute	LC_{50}	0.15	0.0575	**2.6**	100
	Invertebrate, acute (*D.magna*)	EC_{50}	0.0131		**0.2**	100
	Invertebrate, acute (*A. bahia*)	LC_{50}	0.0037		**0.06**	100
	Invertebrate, acute (*H. azteca*)	LC_{50}	0.00017		**0.003**	100
4'OH-deltamethrin (BCS-BY84407)	Fish, acute	LC_{50}	3.99	0.0010	3990	100
	Invertebrate, acute	EC_{50}	670		670000	100
Br_2CA (AE F108565)	Fish, acute	LC_{50}	100000	0.0461	2169197	100
	Invertebrate, acute	EC_{50}	>100000		>2169197	100
Serinyl-BrCA (BCS-CW57835)	Invertebrate, acute	EC_{50}	35300	0.0048	7354167	100
mPBacid	Fish, acute	LC_{50}	13300	0.0044	3022727	100
	Invertebrate, acute	EC_{50}	85000		19318181	100

Bold values do not pass the risk assessment

The acute TER values for all relevant metabolites meet the required trigger of 100 based on FOCUS Step 2 PEC values. Therefore, an unacceptable acute risk to aquatic organisms is not to be expected from these metabolites following the application of deltamethrin EW15 in sugarbeet, cauliflower and wheat.

However, the required trigger is not met for the parent compound deltamethrin, when based on endpoints derived from worst-case laboratory studies. Therefore, TER calculations taking into account higher tier endpoints are provided in the following.

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 10: TER_{LT} calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Sugarbeet					
Deltamethrin	Fish, chronic	NOEC 0.017	0.0690	**0.25**	10
	Invertebrate, chronic (*D. magna*)	NOEC 0.0041		**0.06**	10
	Invertebrate, chronic (*A. bahia*)	NOEC 0.00073		**0.01**	10
	Sediment dweller	NOEC 0.010		**0.14**	10
	Green algae, chronic (*P. subcapitata*)	E_rC_{50} >9100		>131884	10
	Algae, chronic (*N. pelliculosa*)	E_rC_{50} >3.1		>45	10
	Aquatic plants, chronic	EC_{50} >0.779		>11	10
Cauliflower					
Deltamethrin	Fish, chronic	NOEC 0.017	0.0690	**0.25**	10
	Invertebrate, chronic (*D. magna*)	NOEC 0.0044		**0.06**	10
	Invertebrate, chronic (*A. bahia*)	NOEC 0.00073		**0.01**	10
	Sediment dweller	NOEC 0.010		**0.14**	10
	Green algae, chronic (*P. subcapitata*)	E_rC_{50} >9100		>131884	10
	Algae, chronic (*N. pelliculosa*)	E_rC_{50} >3.1		>45	10
	Aquatic plants, chronic	EC_{50} >0.779		>11	10
Spring & winter wheat					
Deltamethrin	Fish, chronic	NOEC 0.017	0.0575	**0.30**	10
	Invertebrate, chronic (*D. magna*)	NOEC 0.0041		**0.07**	10
	Invertebrate, chronic (*A. bahia*)	NOEC 0.00073		**0.01**	10
	Sediment dweller	NOEC 0.010		**0.17**	10
	Green algae, chronic (*P. subcapitata*)	E_rC_{50} >9100		>158261	10
	Algae, chronic (*N. pelliculosa*)	E_rC_{50} >3.1		>54	10
	Aquatic plants, chronic	EC_{50} >0.779		>14	10

The long-term TER values for all relevant metabolites meet the required trigger of 10 based on FOCUS Step 2 PEC values. Therefore, an unacceptable chronic risk to aquatic organisms is not to be expected from these metabolites following the application of deltamethrin EW15 in sugarbeet, cauliflower and wheat.

However, the required trigger is not met for the parent compound deltamethrin, when based on endpoints derived from worst-case laboratory studies. Therefore, TER calculations taking into account higher tier endpoints are provided below.

The following **TER calculations are based on higher tier endpoints as derived from the refined risk assessments for fish and aquatic invertebrates** presented above:

Table 10.2- 11: Refined TER calculations using endpoints derived from higher tier studies (endpoints cover acute and chronic exposure) based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	TER	Trigger
Sugarbeet					
Deltamethrin	Fish [a]	NOEAEC 1.0	0.0690	14	10
	Aquatic invertebrates [b]	NOEAEC 0.023		**0.33**	1
Cauliflower					
Deltamethrin	Fish [a]	NOEAEC 1.0	0.0690	14	10
	Aquatic invertebrates [b]	NOEAEC 0.023		**0.33**	1
Spring & winter wheat					
Deltamethrin	Fish [a]	NOEAEC 1.0	0.0575	17	10
	Aquatic invertebrates [b]	NOEAEC 0.023		**0.4**	1

[a] NOEAEC based on a mesocosm study with the most sensitive fish species (Rainbow trout) that covers both, acute and chronic effects of deltamethrin ([redacted], 2005; M-236605-01-1)

[b] NOEAEC based on the endpoint derived from a lentic freshwater community mesocosm with the formulation Deltamethrin EW15 and other supporting information as explained in the refined risk assessment for aquatic invertebrates

The TER values for fish meet the trigger value based on FOCUS Step 2 PEC values. Therefore, an unacceptable risk to fish is not to be expected following the application of Deltamethrin EW15 in sugarbeet, cauliflower and wheat.

However, the required trigger is not met for aquatic invertebrates in this step. TER calculations using FOCUS Step 3 PEC values are presented below.

Table 10.2- 12: Refined TER calculations using endpoints derived from higher tier studies (endpoint covers acute and chronic exposure) based on FOCUS Step 3

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER	Trigger
Sugarbeet						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0393	D3 (ditch)	**0.59**	1
			0.0016	D4 (pond)	14	
			0.0325	D4 (stream)	**0.71**	
			0.0016	R1 (pond)	14	
			0.0272	R1 (stream)	**0.85**	
			0.0382	R3 (stream)	**0.6**	
Cauliflower						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0476	D3 (ditch)	**0.48**	1
			0.0017	D4 (pond)	14	
			0.0378	D4 (stream)	**0.6**	
			0.0464	D6 (ditch)	**0.5**	
			0.0017	R1 (pond)	14	
			0.0315	R1 (stream)	**0.73**	
			0.0422	R2 (stream)	**0.55**	
			0.0442	R3 (stream)	**0.52**	
			0.0313	R4 (stream)	**0.73**	
Spring wheat						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0401	D1 (ditch)	**0.57**	1
			0.0316	D1 (stream)	**0.73**	
			0.0399	D3 (ditch)	**0.58**	
			0.0014	D4 (pond)	16	
			0.0321	D4 (stream)	**0.72**	
			0.0014	D5 (pond)	16	
			0.0313	D5 (stream)	**0.73**	
			0.0263	R4 (stream)	**0.87**	
Winter wheat						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0400	D1 (ditch)	**0.58**	1
			0.0307	D1 (stream)	**0.75**	
			0.0402	D2 (ditch)	**0.57**	
			0.0332	D2 (stream)	**0.69**	
			0.0399	D3 (ditch)	**0.58**	
			0.0014	D4 (pond)	16	
			0.0317	D4 (stream)	**0.73**	
			0.0014	D5 (pond)	16	
			0.0321	D5 (stream)	**0.72**	
			0.0402	D6 (ditch)	**0.57**	
			0.0014	R1 (pond)	16	
			0.0263	R1 (stream)	**0.87**	
			0.0369	R3 (stream)	**0.62**	
			0.0263	R4 (stream)	**0.87**	

Based on FOCUS Step 3 PEC values, the TER values meet the trigger value only for the pond scenarios. For all other relevant FOCUS scenarios, further refinement using FOCUS Step 4 PEC values is provided below, taking into account a 5 m buffer zone.

Table 10.2- 13: Refined TER calculations using endpoints derived from higher tier studies (endpoint covers acute and chronic exposure) based on FOCUS Step 4 taking into account a 5 m buffer zone

Compound	Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER	Trigger
Sugarbeet						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0128	D3 (ditch)	1.8	1
			0.0137	D4 (stream)	1.7	
			0.0114	R1 (stream)	2.0	
			0.0161	R3 (stream)	1.4	
Cauliflower						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0128	D2 (stream)	1.8	1
			0.0138	D4 (stream)	1.7	
			0.0125	D5 (stream)	1.8	
			0.0115	R1 (stream)	2.0	
			0.0154	R2 (stream)	1.5	
			0.0161	R3 (stream)	1.4	
			0.0114	R4 (stream)	2.0	
Spring wheat						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0109	D1 (ditch)	2.1	1
			0.0114	D1 (stream)	2.0	
			0.0109	D2 (stream)	2.1	
			0.0116	D4 (stream)	1.98	
			0.0113	D6 (ditch)	2.0	
			0.0095	R4 (stream)	2.4	
Winter wheat						
Deltamethrin	Aquatic invertebrates	NOEAEC 0.023	0.0109	D1 (ditch)	2.1	1
			0.0111	D1 (stream)	2.1	
			0.0109	D3 (ditch)	2.1	
			0.0120	D2 (ditch)	1.9	
			0.0109	D2 (stream)	2.1	
			0.0114	D4 (stream)	2.0	
			0.0116	D6 (ditch)	1.98	
			0.0109	D5 (stream)	2.1	
			0.0095	R1 (stream)	2.4	
			0.0133	R3 (stream)	1.7	
			0.0095	R4 (stream)	2.4	

According to the presented risk assessment based on FOCUS Step 4 calculations, the risk to aquatic organisms from the use of the product Deltamethrin EW15 in sugarbeet, cauliflower and wheat is considered acceptable for all scenarios taking into account a **buffer zone of 5 m** to surface water bodies.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report:	**KCP 10.2.1/01, [redacted], [redacted], [redacted]; 2000**
Title:	Acute toxicity to *Oncorhynchus mykiss* (rainbow trout) in a static-renewal system Deltamethrin oil in water emulsion 15 g/L Code: AE F032640 00 EW01 B103
Document No:	M-197428-01-1 (CE00/043)
Guidelines:	- OECD guideline no. 203 - US-EPA Subdivision E, § 72-1 - EU directive 92/69/EWG Annex Part C C.1
GLP:	yes

Objective:
The effect of deltamethrin, oil in water emulsion 15 g/L, on rainbow trout (*Oncorhynchus mykiss*) was tested in a static-renewal system over 96 hours according to OECD guideline 203.

Materials and methods:
Test item: Deltamethrin; oil in water emulsion 15 g/L (analysed a.s. content: 1.48% w/w); code: AE F032640 00 EW01 B103
Three months old trout (average weight: 0.82 g, average length: 3.69 cm) were exposed to the nominal concentrations of 10, 18, 32, 56, and 100 µg test item/L and an untreated control under static-renewal conditions (daily change of test solutions) for 96 hours. Mortality and intoxication symptoms of the test fish were assessed at 24 hour intervals.
Chemical analysis of the freshly prepared and aged (24 hours old) test solutions was performed for the tested concentrations of 10, 32, and 100 µg test item/L. Samples were analysed for the active ingredient deltamethrin using High Performance Liquid Chromatography with ultraviolet detection (HPLC/UV).

Results:
Analytical results:
Analyses of test substance concentrations which were based on deltamethrin revealed that mean measured concentrations over the time of exposure ranged from 75.9% to 83.4% of nominal values for fresh test solutions and ranged from 37.2% to 39.7% of nominal values for aged samples. The time weighted average concentrations of deltamethrin ranged from 54.5% to 56.9% of nominal. Therefore, all concentrations were corrected by the lowest mean measured factor of 0.545. The biological endpoints are based on the following mean measured concentrations: control, 5.45, 9.81, 17.4, 30.5 and 54.5 µg test item/L.

Biological results:
In all treatments spasms of the fish according the release to the new vessels were observed. Although these symptoms were observed on a short term scale only, the concentration without mortality and without any observed effects (NOEC) was set to <5.45 µg test substance/L.

Cumulative mortality [%] during the exposure of Rainbow trout to the test item

Mean measured conc.	24 h	48 h	72 h	96 h
Control	0	0	0	0
5.45 µg test item/L	0	0	0	0
9.81 µg test item/L	0	0	10	40
17.4 µg test item/L	0	0	20	40
30.5 µg test item/L	0	60	100	100
54.5 µg test item/L	90	100	100	100

LC_{50} values for rainbow trout exposed to deltamethrin, oil in water emulsion (15 g/L), based on mean measured concentrations

Test substance:	Deltamethrin, oil in water emulsion 15 g/L
Test object:	Rainbow trout (*Oncorhynchus mykiss*)
Exposure:	96 hours, static-renewal test design (dose-response)
LC_{50} 96 h (95% C.I.):	**14.4 (10.8-19.1) µg test item/L (mean measured)**

Conclusion:
The 96-hour LC_{50} of deltamethrin, oil in water emulsion (15 g/L), based on mean measured concentrations, was determined to be 14.4 µg test item/L.

*****

Report:	**KCP 10.2.1/02; [redacted], [redacted], [redacted]; 2000**
Title:	Acute toxicity to *Daphnia magna* (waterflea) Deltamethrin oil in water emulsion 15 g/L Code: AE F032640 00 EW01 B103
Document No:	M-197398-01-1 (CE00/012)
Guidelines:	OECD No. 202 EU (=EEC) C.2 USEPA (=EPA) E § 72-2
GLP:	yes

Objective:
The acute toxicity of deltamethrin, oil in water emulsion (15 g/L) to the waterflea (*Daphnia magna*) was determined under static conditions according to OECD guideline 202.

Materials and methods:
Test item: Deltamethrin, oil in water emulsion 15 g/L (analysed a.s. content: 1.48% w/w); code: AE F032640 00 EW01 B103
Waterflea (*Daphnia magna*) were exposed under static conditions to nominal concentrations of 0.32, 0.56, 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 µg test item/L and an untreated control. Two replicates with 10 animals each were tested per concentration and control. Chemical analysis of test solutions was performed for the nominal concentrations of 0.32, 0.56, 1.0, 10 and 32 µg test item/L at test intiation and termination. Samples were analysed for the active ingredient deltamethrin by chromatographic determination.

Results:

Analytical results:
Analyses of freshly prepared test solutions revealed test item concentrations ranging from 81% to 122% of nominal values, based on deltamethrin. Analyses of aged samples (48 h) for deltamethrin at experimental termination resulted in test item concentrations ranging from 11.9% to 29.5% of nominal values. The mean measured values over the time of exposure ranged from 48.7% to 68.7%. Therefore all concentrations were corrected by the lowest mean measured factor of 0.487. The biological endpoints are based on the following mean measured concentrations: control, 0.16, 0.27, 0.49, 0.88, 1.56, 2.73, 4.87, 8.77 and 15.6 µg test item/L.

Biological results:

Toxicity of deltamethrin, oil in water emulsion (15 g/L) to *Daphnia magna*:

Mean measured test concentration [µg test item/L]	**Exposed daphnids (=100%)**	**Immobilised daphnids**			
		24 h		**48 h**	
		n	**%**	**n**	**%**
Control	20	0	0	0	**0**
0.16	20	0	0	0	**0**
0.27	20	0	0	0	**0**
0.49	20	0	0	0	**0**
0.88	20	0	0	0	**0**
1.56	20	0	0	15	**75**
2.73	20	0	0	11	**55**
4.87	20	0	0	20	**100**
8.77	20	7	35	20	**100**
15.6	20	13	65	20	**100**

Those daphnids which were still alive in the concentrations of and above 0.88 µg/L moved erratically and/or floated at the water surface. The concentration without any observed effects (NOEC) after 24 and 48 hours was 0.49 µg test item/L.

EC_{50} values for *Daphnia magna* exposed to deltamethrin, oil in water emulsion (15 g/L) based on mean measured concentrations

Test substance:	Deltamethrin, oil in water emulsion, 15 g/L
Test object:	*Daphnia magna*
Exposure:	48 hours, static test design (dose-response)
EC_{50} 24 h (95% C.I.):	12.1 (10.0-15.7) µg test item/L (mean measured)
EC_{50} 48 h (95% C.I.):	**1.33 (0.88-4.87) µg test item/L (mean measured)**

Conclusion:
The 48-hour EC_{50} for immobilization following exposure to deltamethrin, oil in water (15 g/L), in a static test design, was determined to be 1.33 µg test item/L based on mean measured concentrations.

*****

Report:	**KCP 10.2.1/03; [redacted]; 2013**
Title:	Acute toxicity of deltamethrin EW 15B G to the waterflea *Daphnia magna* in a static renewal laboratory test system
Document No:	M-470588-01-1 (EBDAN128)
Guidelines:	EU Directive 91/414/EEC Regulation 1107/2009 Europe US EPA OCSPP 850.1010
GLP:	yes

Objective:
The study was performed, to detect possible effects of the test item on mobility of *Daphnia magna* caused by 48 hours of exposure in a static renewal laboratory test system, expressed as EC_{50} for immobilisation.

Material and methods:
Test item: Deltamethrin EW 15B G, batch 2012-000065, specification No.: 102000025993-01, purity: 1.58% w/w (TOX 09629-00)
Daphnia magna (1st instars < 24 h old, 6 x 5 animals per concentration) were exposed in a static renewal test system for 48 (2 × 24) hours to nominal concentrations of 0, 0.5, 1, 2, 4, 8, and 16 µg test item/L without feeding.
The content of deltamethrin in exposure media was measured for verification of the test item concentrations.

Results:
Analytical results:
The accompanying chemical analysis of deltamethrin in the freshly prepared test solutions at test initiation revealed measured contents between 53% and 91% (mean: 70%) of nominal. The measured concentrations in the aged test solutions at the end of each 24-hour exposure period ranged between 24% and 69% (mean: 40%) of nominal.
No contaminations of deltamethrin were detected in samples from the untreated water control.
Based on the measured a.s. concentrations of deltamethrin (AE F03264) in the test solutions, the corresponding test item concentrations were recalculated and resulted in the following mean measured concentrations: 0, 0.38, 0.65, 0.93, 1.97, 3.56 and 7.61 µg test item/L
All reported biological results were related to mean measured formulation concentrations.

Biological results:

Toxicity of Deltamethrin EW 15 B G to *Daphnia magna*:

Mean measured test concentration [µg test item/L]	Exposed daphnids (=100%)	Immobilised daphnids			
		24 h		48 h	
		n	%	n	%
Control	30	0	0	0	0
0.38	30	0	0	7	23.3
0.65	30	0	0	15	50.0
0.93	30	0	0	17	56.7
1.97	30	2	6.7	28	93.3
3.56	30	2	6.7	29	96.7
7.61	30	14	46.7	30	100

No immobility or other effects on behaviour were observed in the untreated control within 48 hours of exposure.

EC_{50} values for *Daphnia magna* exposed to Deltamethrin EW 15 B G based on mean measured concentrations

Test substance:	Deltamethrin EW 15 B G
Test object:	*Daphnia magna*
Exposure:	48 hours, static-renewal test design (dose-response)
EC_{50} 24 h (95% C.I.):	8.62 (5.93-12.5) µg test item/L (mean measured)
EC_{50} 48 h (95% C.I.):	**0.70 (0.57-0.85) µg test item/L (mean measured)**

Conclusions:
The 48-hour EC_{50} for immobilization following exposure to Deltamethrin EW 15 B G in a static-renewal test design, was determined to be 0.70 µg test item/L based on mean measured concentrations.

*****

Report:	**KCP 10.2.1/04; [redacted], [redacted], [redacted]; 2000**
Title:	Algal growth inhibition - *Pseudokirchneriella subcapitata* Deltamethrin oil in water emulsion 15 g/L Code: AE F032640 00 EW01 B103
Document No:	M-197387-01-1 (CT00/002)
Guidelines:	OECD No. 201 EU (=EEC) 92/69 C.3 USEPA (=EPA) J § 123-2
GLP:	yes

Objective:
A test on growth inhibition of the green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was performed with deltamethrin; oil in water emulsion 15 g/L; under static conditions according to OECD guideline 201.

Materials and methods:
Test item: deltamethrin; oil in water emulsion 15 g/L; purity: 1.48% w/w, Code: AE F032640 00 EW01 B103

Triplicate algal cultures (*Pseudokirchneriella subcapitata*, formerly *Selenastrum capricornutum*) with an initial cell density of 10 000 algal cells/mL were incubated in a synthetic medium at 25 + 1 °C for 96 hours. Nominal test substance concentrations were 1.8, 3.2, 5.6, 10, and 18 mg test item/L with three replicates each, together with an untreated control with six replicates. Samples of the algal populations were removed daily from each test vessel and cell concentrations were determined after 24, 48, 72 and 96 hours test duration. Algal morphology was also observed daily using a light microscope and counting chamber. Chemical analysis of the freshly prepared and aged (96 hours old) test solutions was performed for the active ingredient deltamethrin using High Performance Liquid Chromatography with ultraviolet detection (HPLC/UV). The concentrations were analysed prior dilution.

Results:

Analytical results:

Analyses of freshly prepared test media for deltamethrin revealed concentrations ranging from 57.3% to 106.1% of nominal values. Analyses of samples taken after 48 h resulted in deltamethrin concentrations ranging from 52.0% to 66.9% of nominal values. Analyses for deltamethrin in aged test media at study termination (96 h) resulted in concentrations ranging from 15.4% to 60.7% of nominal values. The mean measured values over the time of exposure ranged from 57.8% to 72.6%. Therefore all concentrations were corrected by the lowest mean measured factor of 0.578. The mean measured concentrations were calculated to be 1.04, 1.85, 3.24, 5.78, and 10.4 mg test item/L. Biological endpoints are based on mean measured values.

Biological results:

Significant inhibition of growth based on a comparison of areas under the growth curves (significance level of alpha = 0.05) was observed in concentrations of 1.04 mg test item/L and above. Significant inhibition of specific growth rate based on a comparison of slopes of the growth curves (significance level of alpha = 0.05) was observed in concentrations of 1.85 mg test item/L and above.

EC_{50} values for *Pseudokirchneriella subcapitata* exposed to deltamethrin, oil in water emulsion (15 g/L) based on mean measured concentrations

Test substance:	Deltamethrin, oil in water emulsion (15 g/L)
Test object:	*Pseudokirchneriella subcapitata*
Exposure:	96 hours, static test design (dose-response)
E_bC_{50} 72 h (95% C.I.):	2.96 (2.92-2.99) mg test item/L (mean measured)
E_rC_{50} 72 h (95% C.I.):	**8.14 (7.99-8.29) mg test item/L (mean measured)**

Conclusion:

The 72-hour E_bC_{50} and E_rC_{50}, based on mean measured concentrations, were determined to be 2.96 mg test item/L and 8.14 mg test item/L, respectively.

*****

Report:	**KCP 10.2.1/05; [redacted] (2011)**
Title:	*Pseudokirchneriella subcapitata* growth inhibition test with deltamethrin EW 15 G
Document No:	M-413217-01-1 (EBDAL035)
Guidelines:	OECD Guideline 201 (2006)
GLP:	Yes (certified laboratory)

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

Materials and Methods:
Test material: Deltamethrin EW 15 G, purity: 1.5% w/w deltamethrin, specified by batch ID: 2010-002975, sample description: TOX08992-00 and specification no.: 102000013165-05.
Pseudokirchneriella subcapitata (freshwater microalgae, formerly known as *Selenastrum capricornutum*) were exposed in a chronic multigeneration test for 3 days under static exposure conditions to nominal concentrations of 0.0960, 0.307, 0.980, 3.13 and 10.0 mg test item/L in comparison to a control. The pH values ranged from 7.8 to 8.2 in the controls and the incubation temperature ranged from 21.4°C to 23.5°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8059 lux.
Quantitative amounts of deltamethrin were measured in all treatment groups and in the control on day 0 and day 3 of the exposure period.

Dates of experimental work: October 01 2010 to December 09 2010

Results:

Analytical results:
The analytical findings of deltamethrin in the treatment levels found on day 0 were 48% to 109% of nominal (average: 82%). On day 3 analytical findings of 65% to 83% of nominal (average: 71%) were found. Therefore, the biological results are based on geometric mean measured concentrations test of the test item (formulated product).

Biological results:

Effects of Deltamethrin EW 15 G on Freshwater Algae (*Pseudokirchneriella subcapitata*) in a 72 h growth inhibition test

Nominal test item concentration [mg test item/L]	Geometric mean measured concentration of test item [mg test item/L]	Cell number after 72 h (means) per mL	(0-72h)-average specific growth rates [days^{-1}]	Inhibition of average specific growth rate [%]
Control	Control	740 000	1.434	--
0.0960	0.0713	627 000	1.379	3.8
0.307	0.181	751 000	1.440	-0.4
0.980	0.733	744 000	1.436	-0.1
3.13	2.74	398 000	1.216	15.2
10.0	8.07	32 000	0.379	73.6

test initiation with 10,000 cells/mL
- % inhibition: Increase in growth relative to the control

No morphological change in algae was observed in any test concentration.

EC_{50} values for *Pseudokirchneriella subcapitata* exposed Deltamethrin EW 15 G based on mean measured concentrations

Test substance:	Deltamethrin EW 15 G
Test object:	*Pseudokirchneriella subcapitata*
Exposure:	72 hours, static test design (dose-response)
E_yC_{50} 72 h (95% C.I.):	2.86 (2.12-4.12) mg test item/L (mean measured)
E_rC_{50} 72 h (95% C.I.):	**5.35 (4.92-5.81) mg test item/L (mean measured)**

Conclusion:
A 72-hour growth inhibition test conducted with Deltamethrin EW 15 on algae (*P. subcapitata)* under static exposure conditions resulted in an E_rC_{50} (0-72 h) of 5.35 mg test item/L based on geometric mean measured concentrations.

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

No new studies were conducted.

CP 10.2.3 Further testing on aquatic organisms

Report:	**KCP 10.2.3/01, [redacted]; 2005**
Title:	Effects of Deltamethrin EW 15 on rainbow trout in aquatic outdoor microcosm enclosures.
Document No:	M-256605-01-1 (ALT JD.2005.1)
Guidelines:	OECD Guidance Document "Freshwater Lentic Field Tests", 2004 (Draft); Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991)
GLP:	yes

Objective:
The aim of the study was to assess the effects of repeated applications of Deltamethrin EW15 in a lentic freshwater ecosystem on the growth and survival of juvenile rainbow trout under outdoor field conditions.

Material and methods:
Test item: Deltamethrin EW 15 (= Decis Protech EW015), purity: 16.24 g deltamethrin/L (1.58% w/w), batch no.: AAIM00846
Juvenile rainbow trout (mean weights and lengths of the groups in the different enclosures ranged from 1.88-2.66 g and 55-62 mm, respectively) were exposed to 4 treatment levels and a control under field conditions for 21 days.
The study was carried out using 10 enclosures in an experimental ditch [redacted], the Netherlands. All enclosures contained approx. 433 dm^3 of water, some macrophytes and had a bottom layer of sediment. The treatment consisted of 3 applications of Deltamethrin EW15 at one week intervals, simulating spray drift. Nominal treatment levels were 125, 250, 500 and 1000 ng a.s./L. Treatments were duplicated, using 2 enclosures per treatment level and 2 controls. The test lasted for 21 days after the first application of the test substance on 11 April 2005. The concentrations of the active ingredient in the water phase were followed over time. Fish mortality and behavior was checked four times per week from day -4 to day 21.The weight and length of the fish were determined 4 days prior to the first application of the test substance (day -4), when they were transferred to the enclosures, and at the end of the experiment (day 21). Dynamics in chlorophyll-a content of phytoplankton, macrophyte species composition and cover and community metabolism (temperature, pH and dissolved oxygen content) were followed over time in all enclosures.

Results:
Analytical results:
The water concentration measured in the enclosures 4 h after the 1st application was on average 88% of the nominal target concentrations. The mean recovery 4 h after the 2nd and 3rd application was 74% of nominal values. The concentration of the test compound decreased steadily after the application with a DT_{50} in water of 0.9 ± 0.2 day (average value over all treatment levels and all applications).
For the nominal treatment levels of 125, 250, 500 and 1000 ng a.s./L, the average peak concentrations were 90, 215, 447 and 1013 ng a.s./L resp., whereas the highest peak concentrations were 109, 224, 478 and 1063 ng a.s./L respectively. Time-weighted average exposure levels over the 21-day treatment period were 16, 37, 96 and 231 ng a.s./L.
As nominal concentrations were confirmed by initial measured concentrations, the biological endpoints are based on nominal values.

Biological results:
No treatment-related effects on macrophytes species composition and cover were observed, nor could any treatment-related effects be demonstrated on the measurement endpoints temperature, oxygen content pH and chlorophyll-a content of phytoplankton.
During the 21-day exposure period to the test substance, 9 out of 100 fish had died and 7 fish were missing at the end of the study. There was no apparent relationship between mortality and the test item concentrations.

Dead and missing fish after 21 days of exposure to deltamethrin

Nominal treatment level [ng a.s./L]	Dead fish [% of inserted]	Fish missing on day 21 [% of inserted]	Sum of dead and missing fish [% of inserted]
0	0 %	21 %	21 %
125	20 %	10 %	30 %
250	15 %	0 %	15 %
500	0 %	6 %	6 %
1000	10 %	5 %	15 %

There were no significant differences in mean length, weight, growth of length and growth of weight of the fish in the various treatment levels.
In the enclosures treated at the highest level (1000 ng a.s./L) several of the fish showed slightly erratic swimming without losing balance. The fish also appeared to be coughing. These symptoms occurred within a few hours after the first and third applications, and were no longer apparent on the next day. Similar behavior was observed in the enclosures treated with 500 ng a.s./L, but only after the first application and not after the second and third application. In view of the fast recovery of the fish within a day after the application these symptoms are considered to be of minor biological relevance.

Conclusion:
At all treatment levels up to and including 1000 ng a.s./L no treatment-related effects were observed on length, weight, growth of length and survival of juvenile rainbow trout. In addition, no consistent treatment-related effects on chlorophyll-a or community metabolism endpoints could be observed after 3 applications of the test substance in a weekly interval. The NOEC is 500 ng a.s./L since the symptoms observed at this test concentration were only short term and were only observed after the first application. Considering the fast recovery of observed symptoms after each application, the No Observed Ecological Adverse Effect Concentration (NOEAEC) can be set at ≥ 1000 ng a.s./L.

Additional information on the analytical methods of this study was provided on request of the RMS in document M-557588-01-1

*****

Report:	**KCP 10.2.3/02, [redacted] 2005**
Title:	Biological Effects and Fate of Deltamethrin EW 015 in Outdoor Mesocosm Ponds.
Document No:	M-246137-01-1 (HBF/BT 07)
Guidelines:	OECD Guidance Document “Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)”, July 2004 (Draft) Guidance Document on Testing Procedures for Pesticides in Freshwater Microcosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991) Community-Level Aquatic System Studies – Interpretation Criteria (2002) (Proceeding from the CLASSIC Workshop)
GLP:	yes

Objective:
The aim of the study was to determine the ecological effects of a repeated simulated drift contamination with Deltamethrin EW 015 on different trophic levels (emergence, zooplankton, macroinvertebrates and phytoplankton) in outdoor mesocosms as an aquatic model ecosystem for lentic aquatic fresh water sytems with different trophic levels. The fate of the compound in the individual compartments (water body and sediment) was monitored simultaneously.

Material and methods:

Test item: Deltamethrin EW 015, purity: 1.64% w/w deltamethrin, batch no.: AAIM00846

Twelve test tanks (6 m^3 water, 1 m water depth) which were used in this study are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approximately 15 cm in height) 7 months prior to the study start. The water was composed of local ground water and water from a nearby uncontaminated pond, which was inoculated several times with zooplankton from a natural pond nearby. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from air borne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before study start. Some weeks before the first application 300 *Asellus aquaticus* were artificially inserted in each pond to establish a stable population of this isopoda. Since the populations could not be maintained in a few ponds during the study, new Asellus aquaticus were added to these ponds. In general, the artificial ponds are representative of a small stagnant water body.

The test substance Deltamethrin EW 015 (Batch.-No.:AAIM00846, AZ No.10459) was applied during the early growing season in May 2004 three times at an interval of 7 days onto the water surface of nine test ponds. The treatment levels were 4.8, 10.5, 23, 51 and 111 ng a.s./L per application (two replicates of 4.8 to 51 ng a.s./L, one replicate for 111 ng a.s./L). Three further tanks were used as untreated controls. The mesocosms were investigated for a period of 14 days before and 105 days after the first treatment (= 91 days after the last treatment). Several times during the study period water and sediment samples were taken and analysed to investigate the concentration of the test substance in water and sediment. Further parameters studied were the taxonomic composition of zooplankton, phytoplankton, macroinvertebrates and emergence of insects at different days before and after the applications. Since Asellus aquaticus was assumed to be one of the most sensitive species in this study, this species was studied intensively in situ on Artificial Substrate Samplers (ASS) and in small cages with leaves which function as traps for these organisms. In addition, bioassays were performed with this species to investigate the potential recovery of a population by immigration of organisms from adjacent water bodies (more details on the setup of the bioassays is given below). The physico-chemical water parameters and the content of chlorophyll-a of phytoplankton were also evaluated, as well as the coverage of the sediment with macrophytes and filamentous algae. One diurnal cycle of oxygen concentration, water temperature and pH was recorded during the study.

Bioassays:

Sixteen bioassays with *Asellus aquaticus* were conducted in the treatment and post-treatment period at thirteen sampling dates in order to demonstrate the potential of a recovery of this species. For a bioassay two 1 L glass bottles per mesocosm were filled with pond water and somer of the exposed leaves from the corresponding pond. The bottles were exposed in a climatised room and slightly aerated. After adaptation of the water samples to the water temperature of the room, ten *Asellus aquaticus* were transferred into each bottle. For the first ten bioassays adult organisms were used. During these studies it became obvious, that these organisms might get too old during the study period according to their seasonal development, since rather high mortalities were observed in the controls as well. Therefore, Therefore, later on two vessels with juveniles and two vessels with adult *Asellus* were used per mesocosm. The experimental time for each bioassay was 21 days with one to two evaluations for survival weekly.

Analytical findings:

The analytical results of water samples taken four hours after each of the three applications show that an average of 94.1% of the nominal concentrations could be found in the mesocosm water confirming nominal concentrations very well. The a.s. disappeared after all applications quickly and steadily with an average half-life in the water column of 22.4 hours. At some sampling dates the percentage of adsorbed active substance in the water was determined, the results revealed that about 2/3 of the total applied amount was bioavailable (solubilized in water) in the pond water, whereas 1/3 was adsorbed to particles as algae or particulate matter.

In the sediment of the 2 lowest test levels (4.8 and 10.5 ng/L) the test substance could be found only once shortly after the first application (limit of detection = 0.03 µg/kg dry weight). The results of the higher test levels (23 to 111 ng/L) show a slight increase of sediment concentrations during about 7 weeks after application resulting up to 20% of total applied amount in the sediment, and a slow decrease during the later part of the study to less than 6% of total applied amount. The DT_{50} for whole system (water plus sediment) is 31.6 hours.

Biological findings:
Direct and indirect effects of the application of deltamethrin to the chemical and physical parameters of the pond water have not been observed at any test concentration. Also no effects on the coverage of the ponds and the biomass of macrophytes and filamentous algae were observed at any treatment level.

Results from the bioassays with *Asellus aquaticus*: All bioassays show a control mortality of 32% on average in three weeks. Nevertheless, all bioassays together indicate clear coherent effects of the pond water and the exposed leaves on survival of *Asellus* shortly (2 to 7 days) after each application. One week after each application at the latest significant effects were not longer detectable.
For the bioassays statistically significant NOEC values of 10.5 to 51 ng a.s./L were calculated at two to seven days after the three applications. The NOEC's were considered consistent, if they occurred on consecutive observation days. Three weeks after the first application (one week after the last application) survival of Asellus in the bioassays was no longer affected by treated pond water and exposed leaves. Thus, assuming only acute effects a potential of full recovery after day 21 was demonstrated.

No-Observed-Effect-Concentrations (ng/L) of *Asellus aquaticus* in the bioassays as obtained from statistical testing ($p < 0.05$, Williams test):

Bioassay No.	Bioassay started on		Observation on day							consistent NOEC
	Date	Day	1-3	4-6	7-9	11-13	14-16	18	21-23	
1 A	05.05.2004	2	23	23	4,8	23	51		51	23
2 A	10.05.2004	7	≥ 111	51	51	51	23	≥ 111	≥ 111	51
3 A	12.05.2004	9	10,5	23	10,5	10,5	10,5		10,5	10,5
4 A	17.05.2004	14	≥ 111	≥ 111	≥111	≥ 111	≥ 111	≥ 111	≥ 111	≥111
5 A	19.05.2004	16	51	23	≥ 111		≥ 111		≥ 111	≥111
6 A	24.05.2004	21	≥ 111	51+	≥ 111	≥ 111	≥ 111		51+	51+
7 A	01.06.2004	29	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
8 A	07.06.2004	35	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
9 A	14.06.2004	42	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
10 A	21.06.2004	49	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
11A	01.07.2004	59		≥ 111		≥ 111			≥ 111	≥ 111
11 J	01.07.2004	59		111		≥ 111			≥ 111	≥ 111
12 A	05.07.2004	63	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
12 J	05.07.2004	63	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
13 A	12.07.2004	70	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111
13 J	12.07.2004	70	≥ 111		≥ 111		≥ 111		≥ 111	≥ 111

A Study conducted with adult organisms
J Study conducted with juvenile organisms
Shaded areas: no data
+ = increase as compared to control findings

Results from the mesocosms: The biological data showed some minor and major effects on some groups of organisms, as indicated in the following Tables. In these Tables, the effects were classified according to the following effect categories according to "Guidance Document on Aquatic Ecotoxicology" in the context of the Directive 91/414/EEC (SANCO 3268/2001 rev.4 (final) of 17 October 2002:

Classification of effects		
1	effect could not be demonstrated	no (statistically significant) effects observed as result of the treatment, and observed differences between treatment and controls show no causal relationship
2	slight effect	effects reported in terms of "slight" or "transient" and/or other similar descriptions, and short-term and/or quantitatively restricted response of sensitive endpoints, and effects only observed at individual samplings
3	pronounced short-term effect	clear response of sensitive endpoints, but total recovery within 8 weeks after the last application, and effects reported as "temporary effects on less sensitive species/endpoints" and/or other similar descriptions, and effects observed at some subsequent sampling instances
4	pronounced effect in short-term study (not relevant in this study)	clear effects (such as strong reductions in densities of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after the (last) application
5	pronounced long-term effect	clear response of sensitive endpoints and recovery time of sensitive endpoints is longer than 8 weeks after the last application, and effects reported as "long-term effects on many sensitive species/endpoints" and/or other similar descriptions, and effects observed at various subsequent samplings.

Effects on species						
		Test concentration [ng a.s./L]				
Zooplankton		4.8	10.5	23	51	111
Phyllopoda						
	Daphnia longispina	1	1	3	3	3
	Simocephalus vetulus	1	1	1	1	2
	Chydorus sphaericus	1	1	1*	2*	2*
	Acroperus harpae	1	1	1	1	2*
	Eurycercus lamellatus	1	1	1	1	1
	Graptoleberis testudinella	1	1	1	1	1
Ostracoda						
	Ostracodes (not det.)	1	1	1	1	1
Copepoda						
	Cyclopoid Copepods	1	1	2	3	3
	Copepod Nauplii	1	2	3	3	3
Rotatoria						
	Keratella quadrata	1	2	3	3	3
	Lecane lunaris	1	1	1	1	2
	Polyarthra spec.	1	1		+	+
	Lepadella patella	1	1	1	+	+
	Asplanchna spec.	1	1	1	+	+
	Trichotria pocillum	1	1	1	1	+
	Synchaeta spec.	1	1	1	1	+
	Testudinella patella	1	1	1	1	1
	Cephalodella spec.	1	1	1	1	1
	Euchlanis deflexa	1	1	1	1	1
Diptera						
	Chaoborus crystallinus larvae	3	3	3	3	3
Taxa richness		1	1	1	1	1
Diversity (Shannon Index)		1	1	2	3	3
Evenness		1	1	1	2	2
Similarity (Steinhaus Index)		1	1	3	3	3
Similarity (Stander's Index)		1	1	3	3	3
Principal Response Curves (PRC)		1	2	3	3	3
Community-NOEC		x				
Lowest population-NOEC		<4.8				
NOEAEC					x[#]	

+ Increase in numbers
* Statistically not significant
[#] The NOEAEC was set to 51 ng a.s./L due to the missing replication at 111 ng a.s./L.

Effects on species						
		Test concentration [ng a.s./L]				
Macroinvertebrates (benthic, ASS)		4.8	10.5	23	51	111
Turbellaria						
	Dugesia spec.	1	1	1	1	1
Oligochaeta						
	Tubificidae	1	1	1	1	1
	Stylaria lacustris	1	1	1	1	1
Hirudineae						
	Helobdella stagnalis	1	1	1	1	1
Diptera						
	Chaoborus crystallinus larvae	see zooplankton evaluation				
	Sum of Chironomid larvae	1	1	1	1	2
Ephemeroptera						
	Cloeon dipterum	1	1	1	1	1
Odonata						
	Ischnura elegans	1	1	1	1	1
Pulmonata						
	Gyraulus albus	1	1	1	1	1
	Radix ovata		1	1	1	1
Taxa richness		1	1	1	1	1
Diversity (Shannon Index)		1	1	1	1	1
Evenness		1	1	1	1	2
Similarity (Steinhaus Index)		1	1	1	1	1
Similarity (Stander's Index)		1	1	1	1	1
Principal Response Curves (PRC)		1	1	1	2	2
Community-NOEC				x		
Lowest population-NOEC (**)					x	
NOEAEC					x[#]	

	Test concentration [ng a.s./L]				
Macroinvertebrates (benthic)	4.8	10.5	23	51	111
Oligochaeta					
Tubificidae		1	1	1	1
Diptera					
Chironomidae larvae	1	1	1	1	1
Ceratopogonidae larvae	1	1	1	1	1
Pulmonata					
Gyraulus albus	1	1	1	1	1
Bivalvia					
Pisidium spec.	1	1	1	1	1
Taxa richness	1	1	1	1	1
Diversity (Shannon Index)	1	1	1	1	1
Evenness	1	1	1	1	1
Similarity (Steinhaus Index)	1	1	1	1	1
Similarity (Stander's Index)	1	1	1	1	1
Principal Response Curves (PRC)	1	1	1	1	1
Community-NOEC				x[#]	
Lowest population-NOEC				x[#]	
NOEAEC				x[#]	

[#] The NOEAEC was set to 51 ng a.s./L due to the missing replication at 111 ng a.s./L.

Effects on species						
		Test concentration [ng a.s./L]				
Emergence		4.8	10.5	23	51	111
Chironomidae						
	Sum of Chironominae	1	1	1	1	2
	Chironominae (female)	1	1	1	1	2
	Chironomus spec. (male)	1	1	1	1	3
	Dicrotendipes spec. (male)	1	1	1	1	1
	Paratanytarsus spec. (male)	1	1	1	1	1
	Einfeldia spec. (male)	1	1	1	1	1
	Micropsectra spec. (male)	1	1	1	1	1
	Cryptotendipes spec. (male)	1	1	1	1	1
	Polypedilum spec. (male)	1	1	1	1	1
	Sum of Orthocladiinae	1	1	1	1	1
	Orthocladiinae (female)	1	1	1	1	2
	Orthocladiinae (male) cf. Dratnalia sp.	1	1	1	1	1
	Cricotopus spec. (male)	1	1	1	1	1
	Psectrocladius spec. (male)	1	1	1	1	1
	Limnophyes spec. (male)	1	1	1	1	1
	Corynoneura spec. (male)	1	1	1	1	1
	Acricotopus spec. (male)	1	1	1	1	1
	Sum of Tanypodinae	1	1	1	1	1
	Tanypodinae (female)	1	1	1	1	2
	Tanypus spec. (male)	1	1	1	1	1
	Ablabesmyia spec. (male)	1	1	1	1	1
	Holotanypus spec. (male)	1	1	1	1	1
	Psectrotanypus spec. (male)	1	1	1	2	2
	Monopelopia spec. (male)	1	1	1	1	1
Culicidae						
	Anopheles spec.	1	1	1	1	1
Chaoboridae						
	Chaoborus crystallinus	2	2	2	2	2
Ephydridae						
	Clanoneurum spec.	1	1	1	1	1
Ephemeroptera						
	Cloeon spec.	1	1	1	1	1
Taxa richness		1	1	1	1	2
Diversity (Shannon Index)		1	1	1	1	2
Evenness		1	1	1	1	1
Similarity (Steinhaus Index)		1	1	1	1	1
Similarity (Stander's Index)		1	1	1	1	1
Principal Response Curves (PRC)		1	1	1	2	2
Community-NOEC				x		
Lowest population-NOEC		< 4.8				
NOEAEC					x[#]	

[#] The NOEAEC was set to 51 ng a.s./L due to the missing replication at 111 ng a.s./L.

Effects on species					
	Test concentration [ng a.s./L]				
	4.8	10.5	23	51	111
Diatomeae	1	1	1	1	2
Nitzschia spec.	1	1	1	1	2
Cryptophyceae	1	1	1	1	2
Chroomonas spec. <10 µm	1	1	2	2	2
Cryptomonas spec. 10-20 µm	1	1	1	1	2
Cryptomonas spec. 30-40 µm	1	1	1	1	1
Englenophyta	1	1	1	1	1
Conjugatophyceae	1	1	1	1	1
Cosmarium spec.	1	1	1	1	1
Cyanobacteria (Merismopedia spec.)	1	1	1	1	1
Sum of filamentous algae	1	1	1	1	1
Taxa richness	1	1	1	1	1
Diversity (Shannon Index)	1	1	1	1	1
Evenness	1	1	1	1	1
Similarity (Steinhaus Index)	1	1	1	1	2
Similarity (Stander's Index)	1	1	1	1	1
Principal Response Curves (PRC)	1	1	1	1	1
Community-NOEC			x		
Lowest population-NOEC		x			
NOEAEC				x[#]	

	Test concentration [ng a.s./L]				
	4.8	10.5	23	51	111
Asellus aquaticus					
Asellus in mesocosms					
Leaf cages	1	1	3	3	3
ASS	1	1	3	3	3
Leaf cages and ASS	1	1	3	3	3
Asellus bioassay					
Bioassay 1 (Day 2)	1	1	1	2	3
Bioassay 2 (Day 7)	1	1	1	1	2
Bioassay 3 (Day 9)	1	1	2	3	3
Bioassay 4 (Day 14)	1	1	1	1	1
Bioassay 5 (Day 16)	1	1	1	2*	2
Bioassay 6-13 (Day 21 to Day 70)	1	1	1	1	1
Lowest In situ-NOEC		x			
Lowest bioassay-NOEC		x			
NOEAEC				x[#]	
	Test concentration [ng a.s./L]				
Phytoplankton	4.8	10.5	23	51	111
Chlorophyceae	1	1	1	1	1
Scenedesmus spec.	1	1	1	1	+
Schroederia spec.	1	1	1	1	1

+ Increase in numbers
* Statistically not significant
[#] The NOEAEC was set to 51 ng a.s./L due to the missing replication at 111 ng a.s./L.

At the end of the study, no **zooplankton** taxon showed significant differences in abundance compared to controls, demonstrating the recovery of the zooplankton after the third application within 7 weeks. Chaoborus crystallinus was identified as the most sensitive zooplankton taxon with consistent effects even at 4.8 ng a.s./L immediately after application until about 2 weeks after the last application when a full recovery of the *Chaoborus* population was observed. The crustaceans, especially the copepods and *Daphnia longispina*, proved to be the next most sensitive zooplankton group exhibiting a consistent NOEC of 4.8 ng a.s./L and 10.5 ng a.s./L, respectively. The rotifers were either suppressed (especially *Keratella quadrata*, consistent NOEC of 4.8 ng a.s./L) or promoted (e.g. *Polyarthra* spec., consistent NOEC of 10.5 ng a.s./L) obviously by secondary effects. The PRC (Principal Response Curve) and to some degree Similarity and Shannon Diversity Indices reflected these effects on the zooplankton with a community NOEC of 4.8 ng a.s./L. However, all effected populations recovered shortly after the last application and reached control abundances within some weeks only at all treatment levels including the highest one. Seven weeks after the last application, no taxon showed significant differences in abundance compared to controls, demonstrating the full recovery of the zooplankton community. Due to the missing replication at 111 ng a.s./L the results of this study yield a NOEAEC (no observed ecological adverse effect concentration) of 51 ng a.s./L for the zooplankton.

In **sediment**, significant impacts on the identified species in the ASS (Artificial Substrate Samplers) were only obtained for chironomid larvae at the highest test concentration, resulting in a NOEC of 51 ng a.s./L for the evenness and 23 ng a.s./L for the PRC, whereas all other community parameters did not indicate any effect up to the highest test concentration. Observed effects were even short-term only: no long-lasting effects could be detected. In the sediment samples, no effects even up to the highest treatment level could be detected (NOEC 111 ng a.s./L).

Direct effects of the test item on the **emergence** of some insects were detected for five taxonomic groups: *Chironomus* spec., Orthocladiinae, *Psectrotanypus* spec., Tanypodinae (females) and *Chaoborus crystallinus*. Except of *Chaoborus* (same NOEC as in zooplankton), all other groups were affected only at the highest treatment level (*Psectrotanypus* spec. also at 51 ng a.s./L), with a full recovery within 8 weeks after last application for the latest for all species. Thus, the community indices yielded a community NOEC of 23 ng a.s./L. Because of the fast and full recovery in emergence (which even included the full aquatic life cycle of the emerged insects) within the first weeks after application on the one hand and the missing replication of the highest treatment level of 111 ng a.s./L on the other hand, the NOEAEC for emergence can also be set as 51 ng a.s./L.

In the mesocosms clear effects on ***Asellus aquaticus*** were demonstrated for the 3 highest test concentrations both in leaf cages and ASS. At 10.5 ng a.s./L *Asellus* was only short-term affected after the first application indicating only a decrease in mobility but no mortality. (Both sampling methods indicate a reduction in activity of individuals, which does not necessarily mean mortality). Thus, a consistent NOEC of 10.5 ng a.s./L can be derived from this study for *Asellus* in the mesocosms. In the 3 highest test concentrations the abundance of *Asellus* reached mostly the level of controls until study termination. After day 70 the proportion of juveniles in the higher treated ponds reached the level of controls. A full recovery to control level within 8 weeks after last application could not be demonstrated for 23, 51 and 111 ng a.s./L. However, the differences between control and treatment levels are small and population abundances clearly increased in these ponds, as demonstrated by the increasing number of juvenile organisms and the corresponding reproduction in situ.
The bioassay findings confirm that water and food samples from the mesocosms taken at the latest one week after the applications did not have any negative effects on *Asellus aquaticus*. Overall, the NOEAEC for *Asellus* was 51 ng/L due to the missing replication at 111 ng a.s./L.

No direct toxic effects were observed on the **phytoplankton**. During the application period cell densities of some species, as e.g. the dominant *Chroomonas* spec., were slightly lower at higher treatment levels for a short time than in the controls caused by indirect food web effects, probably by toxic effects of the

test item treatments on the copepod populations, which enhanced the rotifer population density by decreased competition. The community NOEC for phytoplankton was 23 ng a.s./L and the NOEAEC 51 ng a.s./L because of the missing replication at the highest treatment level.

Conclusion:
The fate of deltamethrin demonstrates a steady and fast decline of deltamethrin in the mesocosm water with a mean DT_{50} of 22.4 hours, and a mean DT_{50} of 31.6 hours for the whole test system (water plus sediment). In the sediment of the 2 lowest test concentrations (4.8 and 10.5 ng a.s./L) the active substance was only detected once shortly after application. The results of the higher test concentrations (23 to 111 ng a.s./L) show a slight increase of the amount of the test substance in the sediment for about the first 7 weeks after application and a slow but constant decrease thereafter.

Chaoborus crystallinus was identified as the most sensitive taxon with consistent effects even at 4.8 ng a.s./L immediately after application until about a very few weeks after the last application when a full recovery had been observed even at the highest test level. At 10.5 ng a.s./L also short-term effects for one Rotatoria species (*Keratella quadrata*) and Copepod Nauplii had been observed. *Asellus aquaticus* showed just a reduced activity at this test level for a very few days after application without any sign of mortality or affected reproduction. At 23 and 56 ng a.s./L effects on 1 to 3 individual more species had been observed, but also these effects were short-term only with a full recovery within the first weeks after the last application. The abundance of *Asellus* was clearly reduced after application at this test levels but reached mostly the level of controls until study termination. The differences between control and treatment levels were small and population abundances clearly increased in these ponds during the study, as also demonstrated by the increasing number of juvenile organisms. The bioassay findings confirm that water and food samples from the mesocosms taken at the latest 1 week after the applications did not have any negative effects on *Asellus aquaticus*. At 111 ng a.s./L the number of affected zooplankton and insect species was distinctly higher, and the effects on *Asellus aquaticus* even more developed as compared to lower treatment levels.

Based on these findings and because of the missing replication at the highest test level, 51 ng a.s./L can be concluded as the overall NOEAEC (no observed ecological adverse effect concentration) of this study.

Notifier's comment:
Although the author/study director derived a higher NOEAEC in his report, a NOEAEC of 23 ng a.s./L is considered for this study in the risk assessment for aquatic invertebrates as a more conservative approach. This is also in line with an independent evaluation from [redacted] (2005; M-254687-01-1).

*****

Report:	**KCP 10.2.3/03, [redacted]; 2005**
Title:	Bioassay on the Effects of Deltamethrin EW 15 on *Gammarus pulex* in mesocosm water
Document No:	M-246173-01-1 (HBF/BT 08)
Guidelines:	OECD Guidance Document "Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms)", July 2004 (Draft) Guidance Document on Testing Procedures for Pesticides in Freshwater Mesocosms (SETAC-Europe Workshop, Monks Wood, UK, July 1991) Community-Level Aquatic System Studies - Interpretation Criteria (2002) (Proceedings from the CLASSIC Workshop)
GLP:	Yes

Objective:

The aim of the study was to run a bioassay in order to demonstrate a potential recovery of *Gammarus pulex* populations parallel to a mesocosm study on Deltamethrin ([redacted] 2005; M-246137-01-1). This allows a better control and evaluation of the effect of Deltamethrin on *Gammarus pulex* as a direct test method for *Gammarus* in the mesocosms, which prefer natural habitats with running water instead of the lentic conditions of the mesocosm study.

Material and methods:

Test item: Deltamethrin EW 015, purity: 1.64% w/w deltamethrin, batch no.: AAIM00846; TOX-No. AZ 10459

In this study the ecological effects of Deltamethrin EW 15 were studied on the aquatic invertebrate *Gammarus pulex*. The investigation was performed within several consecutive bioassays running parallel to the mesocosm study ([redacted] 2005, M-246137-01-1). The bioassay test water and food for the Gammarids (*Populus* leaves) originated from the mesocosm study with deltamethrin, the test organisms from a laboratory culture.

The test regime enabled the investigation of the toxicity of deltamethrin to *Gammarus pulex* and demonstration of the possibility of a population recovery by immigration of new individuals into an affected system.

The 12 test tanks (6 m² water, 1 m water depth) used in the mesocosm are especially designed systems which allow the establishment of almost identical conditions at the start of a study. The bottoms of the artificial tanks were covered with natural sediment (approx. 15 cm in height) 7 months prior to beginning of the study. The water was composed of local ground water and water from an uncontaminated pond nearby, which was inoculated several times with zooplankton also from a natural pond nearby. Natural communities developed spontaneously from seeds and roots of aquatic plants as well as from airborne and naturally transferred stages of planktonic, benthic and filamentous algae organisms during the months before the beginning of the study. In general, the artificial ponds were representative of a small stagnant mesotrophic water body.

The test substance was applied during the early growing season in May 2004, three times at an interval of 7 days onto the water surface of 9 test ponds. The treatment levels were 4.8, 10.5, 23, 51 and 111 ng a.s./L per application (two replicates 4.8 to 51 ng a.s./L, one replicate for 111 ng a.s./L). Three further tanks were used as untreated controls.

The test organisms (*Gammarus pulex*) were derived from ditches of the research institute [redacted] (The Netherlands). They were cultured in the laboratory at about 12-15 °C, LD 16:8 hours in aerated tanks and fed by leaves of *Populus spec.*
Two and 7 days after each application (and 4 hours after the 2nd and 3rd application), and on days 15, 21 and 28 after the last application of the mesocosms, pond water samples were taken from the mesocosms, together with some of the exposed leaves (*Populus spec.).* The bottles were exposed in a climatised room (same climatic conditions as the culture) and slightly aerated. After adaptation of the water samples to room temperature within a few hours, ten *Gammarus pulex* of similar size were transferred from the culture into each bottle. The experimental time for each bioassay was 3 weeks with 1 to 2 evaluations weekly. Surviving and dead animals were counted to calculate the survival rate. Water and living animals were refilled into the test bottles each time.
Univariate analyses were performed to calculate NOEC values.

Findings:
A response from *Gammarus pulex* could only be observed at the highest treatment levels of 51 ng a.s./L and 111 ng a.s./L. A reduction of the numbers of surviving Gammarids was noted at these concentrations in the bioassay water and food samples taken 4 hours to 2 days after application. Nevertheless, no effects were found at any of the test concentrations, even the highest one, in bioassays established seven or more days after applications.

Table 10.2.6- 1: Calculated NOEC values after each application

	NOEC (ng a.s./L) after		
Time after application	1st application	2nd application	3rd application
4 hours	not tested	23	23
2 days	23	23	≥ 111
7 days	≥ 111	111	≥ 111
15 days	see 2nd application	see 3rd application	≥ 111
21 days			≥ 111
28 days			≥ 111

Conclusion:
A NOEC of 23 ng a.s./L can be derived from this bioassay study. At higher test concentrations, mortality was observed only in samples taken during the first 2 days after application. Samples taken thereafter did not indicate any toxic effects, even at the highest test concentration of 111 ng a.s./L.

Further information concerning the validation data of this study was provided on request of the RMS in document M-588250-01-1

*****

Report:	**KCP 10.2.3/04, [redacted] (2005)**
Title:	Evaluation report on higher-tier tests to assess the ecological risks of the insecticide deltamethrin to freshwater organisms
Document No:	M-254687-01-1
Guidelines:	Does not apply
GLP	no

In the context of this evaluation report, the author assessed the treatment-related effects observed in the mesocosm studies of [redacted] (2001; M-200619-03-1) and [redacted]. (2005; M-246137-01-1). Responses of the measurement endpoints were considered treatment-related when:

4. Clear concentration-response relationships were evident that could not be observed during the pre-treatment period
5. Statistically significant effects were demonstrated on at least two consecutive sampling dates
6. The statistically significant effects were ecologically relevant.

In this report the observed treatment-related responses in the microcosms are evaluated by using "Effect classes" adapted after [redacted] et al. (2000)[4], viz.:

Class 1: No effects demonstrated
No consistent adverse effects are observed as a result of the treatment. Observed differences between treated test systems and controls do not show a clear causality.

Class 2: Slight effects
Confined responses of sensitive endpoints (e.g., partial reduction in abundance). Effects observed on individual samplings only and/or of a very short duration directly after treatment.

Class 3a: Clear short-term effects, lasting < 8 weeks
Pronounced direct or indirect effects on measurement endpoints. Recovery takes place within eight weeks post final treatment and the total time span of effects (sum of response periods after each treatment) does not exceed 8 weeks (56 days). Transient effects reported on both sensitive and less sensitive endpoints. Effects observed on a sequence of samplings.

Class 3b: Clear short-term effects, lasting > 8 weeks, but full recovery within 8 weeks post last application
Convincing direct or indirect effects on measurement endpoints and recovery of these endpoints within 8 weeks after the last treatment, but the total time span of effects (including the period between treatments) is larger than 8 weeks (56 days). Effects reported over a sequence of samplings.

Class 4: Pronounced effects in short-term study
Not relevant for the evaluated study.

Class 5a: Clear long-term effects, but full recovery before the end of the experiment
Convincing effects on measurement endpoints in the period after 8 weeks post last application but full recovery of these endpoints observed before termination of the study.

Class 5b: Clear long-term effects, without full recovery
Convincing effects on measurement endpoints also in the period after 8 weeks post last application and without full recovery before termination of the study.

An overall summary of the "effect classes" determined by the author for several categories of endpoints is given in the tables below. Within each category the most sensitive endpoint was selected. In this evaluation report the author considers class 3 effects acceptable to derive the NOEAEC.

[4] [redacted], R.P.A. van Wijngaarden & G.J. van Geest (2000): Ecological risks of pesticides in freshwater ecosystems. Part 2: Insecticides. Alterra-rapport 089, 142 pp.

Summary table of study from Schanné & Van der Kolk, 2001:

Nominal peak concentration	1.0 ng/L	3.2 ng/L	10 ng/L	18 ng/L	32 ng/L	56 ng/L	100 ng/L	180 ng/L
Micro-Crustacea	1	1	3a	5a	5a	5b	5b	5b
Other zooplankters	1	1	1	1	3a *	3a *	3a *	3a *
Macro-Crustacea	2	?	3b	5b	5b	5b	5b	5b
Insects	2	3a	3a	3b	3b	3b	5b	5b
Other macro-invertebrates	1	1	1	1	1	3a *	3b *	3b *
Water quality endpoints	1	1	1	1	1	1	1	1

* = responses can at least in part be explained as resulting from indirect effects

The study of [redacted] (2001) revealed class 2 effects on two very sensitive taxa only at 1 ng deltamethrin/L, the lowest treatment-level tested (see table above). Since these effects were transient and slight, the overall $NOEC_{community}$ of the study is set in this evaluation report at 1 ng deltamethrin/L. When considering class 3a/3b effects (clear short-term effects) on a few populations of crustaceans (*Asellus*, *Daphnia*) and insects (*Chaoborus*, Ephemeroptera) acceptable a NOEAEC of 10 ng deltamethrin can be derived from the study. At treatment-levels of 18 ng deltamethrin/L and higher the results revealed clear long-term effects (class 5a/5b) on calanoid copepods and on *Asellus aquaticus*.

Summary for study of [redacted] 2005:

Nominal peak concentration	4.8 ng/L	10.5 ng/L	23 ng/L	51 ng/L	111 ng/L
Micro-Crustacea	1	3a	3a	3a	3a
Other zooplankters	2 *	2 *	3a *	3a *	3a *
Macro-Crustacea	1	2	3b – 5a? #	5a - 5b?	5b?
Insects	3a	3a	3a	3a	3b – 5b
Other macro-invertebrates	1	1	1	2 *	2 *
Phytoplankton	1	1	3a *	3a *	3a *
Water quality endpoints	1	1	1	1	1

* = responses can at least in part be explained as resulting from indirect effects
# = The in situ bioassays show that potential recovery may be fast

An overall $NOEC_{community}$ cannot be derived from the experimental pond study provided by [redacted] (2005), since the lowest treatment-level (4.8 ng deltamethrin/L) resulted in class 3a effects on the phantom midge *Chaoborus*. At treatment-levels of 23 ng deltamethrin/L and higher, long-term effects on *Asellus aquaticus* cannot be excluded on basis of the responses observed in the microcosm study of [redacted] (2005). In situ bioassays performed with *Asellus aquaticus* and water from the outdoor microcosms, however, show that potential recovery of this isopod may be fast. For this reason a NOEAEC of 23 ng deltamethrin/L can be derived when both the outdoor microcosm study and the in situ bioassays are considered.

Taking into account the two mesocosm studies and related bioassays, the author comes to the following conclusions:

- The two outdoor semi-field tests reported by [redacted] (2001) and [redacted] (2005) can be used to evaluate the effects of short-term pulsed (3x, interval 7 d) deltamethrin exposure on freshwater communities.
- The study of [redacted] (2001) used test systems that had a relatively high diversity of freshwater arthropods. In this study relatively worst case exposure conditions were simulated, due to mixing of the test compound in the water column immediately after deltamethrin application.
- The study of [redacted] (2005) is characterised by test systems with a lower (but not exceptional for such model ecosystem studies) diversity of freshwater arthropods. However, several very sensitive arthropod populations (e.g. *Chaoborus*, *Asellus*) were present and additional bioassays with the sensitive macro-crustaceans *Asellus aquaticus* and *Gammarus pulex* were performed. In addition, the study of [redacted] (2005) more realistically simulated the risks due to spray drift and described the stratification and dynamics in exposure concentrations in the course of the experiment in great detail.
- On basis of the most sensitive endpoints studied a $NOEC_{community}$ of approximately 1 ng deltamethrin/L can be derived from the study of [redacted] (2001).
- Under the assumption that short-term (class 3) effects on a few populations of sensitive arthropods are acceptable a NOEAEC of approximately 10 ng deltamethrin/L (based on nominal initial concentration) can be derived from the semi-field experiment reported by [redacted] (2001), and of 10 - 23 ng deltamethrin/L for the study reported by [redacted] (2005).
- Publications on the ecological effects of other pyrethroids in aquatic micro/mesocosms suggest that the NOEAEC of approximately 10 - 23 ng deltamethrin/L as observed in the studies reported by [redacted] (2001) and [redacted] (2005) can be used as an Environmentally Acceptable Concentration of deltamethrin in freshwater ecosystems (without applying an extra Uncertainty Factor), at least if short term effects on a few insects and crustaceans are considered acceptable.

*****

Report:	**KCP 10.2.3/05, [redacted] (2007)**
Title:	Analysis and interpretation of the zooplankton dynamics after application of Deltamethrin EW 015 to aquatic mesocosms with special focus on the *Chaoborus crystallinus* population
Document No:	M-291864-01-1 (HBF/BT 07)
Guidelines:	Does not apply
GLP	no

Summary:

Based on the results of the mesocosm study with Deltamethrin EW 15 ([redacted] 2005; M-246137-01-1) a food web evaluation of the mesocosm zooplankton community has been used to analyse direct and indirect effects on the zooplankton composition observed in the above mentioned mesocosm

study.

Exemplary life-cycle calculations, observations and considerations on the population densities and emergence of ***Chaoborus crystallinus*** in the mesocosm revealed that this population probably recovered by means of external sources via egg masses laid on the water surface of treated ponds soon after the last application. Larvae which hatched from egg masses about 6 to 7 days onwards after the last application of 4.8 ng a.s./L (7 to 8 days at 111 ng a.s./L; egg deposition about 4 days earlier) survived and got trapped as emerged midges later on during the study.

Following the food web evaluation it is seen as highly improbable that the test item had any effects on **rotifers**. On the contrary, the population growth of rotifers was promoted due to an indirect effect via the toxicant-induced loss of effective predators (*Chaoborus*) and of competing Cladocerans (*Daphnia longispina*, *Chydorus sphaericus*), until the predators came into play again: *Asplanchna* and new young chaoborid larvae repopulating the mesocosms probably caused the sharp decline in rotifers soon after the applications. Thus, it can be confirmed that all observed effects on the population dynamics of rotifers are to be considered as secondary effects of the treatment with Deltamethrin.

The increase in the population densities of ***Daphnia longispina*** at higher test concentrations some weeks after the applications does probably also not depend on the test item concentrations. Taking into consideration the rapid dissipation of the test item from the water phase and the short generation cycles (< 10 d) of this species, the start of recovery appears rather delayed. Since the daphnid densities did not reach control densities until the first emergence of the chaoborids, most probably the growing population of 3rd- and 4th-instar larvae substantially contributed to the delayed recovery by predation.

With respect to the **copepod** populations the author shares the description and interpretation of [redacted] (2005; M-246137-01-1) that the toxic effect of the test item on the cyclopoid copepods (juvenile copepodids plus adult copepods) was slightly lower than on the nauplii (only at test concentration of 23 to 111 ng a.s./L), but the population density reached the control level not before day 29. However, the effects on the nauplii are seen by the present author to be also caused by the decline of the copepods themselves, since their decline caused less production of eggs and thus nauplii.

Overall, *Chaoborus crystallinus* was found as the most sensitive species in this mesocosm study with Deltamethrin, demonstrating a distinct reduction in abundance of larvae and emerging midges immediately after the application at all treatment levels. However, larvae hatching from egg masses in the treated pond of the highest test level (111 ng a.s./L) already survived 7 to 8 days after the last application and emerged later on. In addition the abundance of *Daphnia longispina* and copedopds (mainly nauplii) was affected by Deltamethrin at the highest test levels. Although the recovery for *D. longispina* was delayed by the predation of a growing population of *Chaoborus* larvae, the populations of both, *Daphnia longispina* and copedopds (mainly nauplii) recovered even up the highest test level within some weeks after the last application at the latest. The population dynamics of *Chaoborus crystallinus* also caused some short-term indirect food web effects (as on rotifers and phytoplankton). Thus, the treatment with deltamethrin caused distinct short-term effects on a few zooplankton species, which also induced fluctuations on other zooplankton and phytoplankton species within the food web for some weeks only.

*****

The following study was conducted with the mixed formulation Thiacloprid & Deltamethrin OD 100+10. As the observed effects on aquatic invertebrates, especially *Asellus aquaticus*, seem to be driven mainly by the deltamethrin content in the formulated product, the results are considered in the risk assessment as supplemental information.

Report:	**KCP 10.2.3/06, [redacted], [redacted] [redacted] [redacted] 2005**
Title:	Fate and effects of Thiacloprid & Deltamethrin OD 100 + 10 in outdoor mesocosm ponds.
Document No:	M-259938-01-2 (BAY-018/4-52)
Guidelines:	Guidance Document on Higher Tier Aquatic Risk Assessment for Pesticides (HARAP), SETAC Europe, 1999 European Commission. Health & Consumer Protection Directorate-General. Directorate E1 – Plant Health. (2002): Working Document, Guidance Document on Aquatic Ecotoxicology in the context of Directive 91/414/EEC, 17 October 2002 Community Level Aquatic System Studies – Interpretation Criteria (CLASSIC Workshop), SETAC 2002 OECD Guidance Document (2003): Draft Guidance Document on Simulated Freshwater Lentic Field Tests (Outdoor Microcosms and Mesocosms), August 2003
GLP:	Yes (certified laboratory)

Objective:
The aim of the study was to determine the ecological effects of a simulated drift contamination with Thiacloprid & Deltamethrin OD 100+10 on different trophic levels (emergence, zooplankton, macroinvertebrates and phytoplankton) in a lentic aquatic freshwater system. At the same time the fate and distribution of the compound in the individual compartments (water body, sediment) was monitored. *Asellus aquaticus*, one of the acutely most sensitive invertebrate species, was artificially introduced about three weeks before application into the mesocosm and monitored. In addition *Asellus* was tested parallel in laboratory bioassays using mesocosm water.

Material and methods:
Test item: Thiacloprid & Deltamethrin OD 100 + 10, purity: 9.8% w/w thiacloprid and 0.938 % w/w deltamethrin, batch no: 08136/0061(0020)

Twelve cylindrical ponds (diameter 2.5 m; total depth 1.5 m, water depth above sediment surface: 1 m; surface area: 4.91 m²) were used in this study. The ponds were linked by a system of pipes which allow the establishment of almost identical conditions at start of the study. The bottoms were covered with natural sediment (approximately 10 cm in height) five months prior to the study start. The sediment was covered with local tap water. The mesocosm water was additionally stocked with phytoplankton and zooplankton organisms from field collections from several local non-polluted ponds. *Asellus aquaticus*, one of the acutely most sensitive invertebrate species was artificially introduced three weeks before the application and monitored. In replicates, where the *Asellus* population was severely affected two weeks after the application (one replicate of the 1.1 µg/L concentration, all replicates from higher concentrations), additional *Asellus* were introduced on days 12, 47 and 57 (each time 200 individuals per replicate) to demonstrate a potential recovery of the isopoda populations.
The test item Thiacloprid & Deltamethrin OD 100+10 was applied once during the early growing season on 12 May 2004 onto the water surface of nine mesocosms. The treatment levels were 0.5, 1.1, 2.5, 5.5, and 11.9 µg test item/L corresponding to 50.4, 110, 242, 536, and 1166 ng thiacloprid/L and 4.8, 10.5,

23, 51, and 111 ng deltamethrin/L. Two replicates were used for all except the unreplicated highest treatment level. Three mesocosm served as untreated controls.

The mesocosms were investigated 14 days before and 77 days after the treatment. Several times during the study period water and sediment samples were taken and analyzed to investigate the concentration of the active ingredients in water and sediment. Further parameters studied were the taxonomic composition of zooplankton, phytoplankton, macroinvertebrates and emergence of insects at different days before and after application of the test item. Since *Asellus aquaticus* was assumed to be one of the most sensitive species in this study this species was investigated in situ on Artificial Substrate Samplers (ASS) and in small cages with leaves which function as traps for these organisms. Additionally, laboratory bioassays were performed with this species to investigate the potential recovery of a population by migration of organisms from adjacent water bodies. Physico-chemical parameters, the chlorophyll-a content of the phytoplankton, coverage of the sediment with macrophytes and the thickness of the periphyton layer on the mesocosm walls were also evaluated. Two diurnal cycles of conductivity, oxygen concentration, water temperature and pH were recorded during the study.

Analytical findings:
The analysis of all spray solutions show an average of 105% for the active substance deltamethrin and 97.1% for thiacloprid confirming nominal concentrations.
The analytical results of water samples taken four hours after the application show a distinct stratification for both active substances. However, after 24 hours deltamethrin and thiacloprid were distributed homogenously in the whole water column. Deltamethrin disappeared quickly after application with an average half-life in the water column of 2 days. The DT_{50} for thiacloprid was calculated to be 43 days.
In the sediments of the two lowest treatment levels deltamethrin was not found during the study. In the higher test levels concentrations increased up to about day 42 and decreased during the second half of the study. Thiacloprid was found in all treated sediments and showed a slower increase in the sediment during the study period up to about day 49 with a decrease until the end.
For the total system, the DT_{50} were slightly higher than for the water (2.5 and 49 d, respectively). At the end of the study (105 days after application), deltamethrin was found only in the sediment of the mesocosms of the two highest treatment levels with 7% and 1% of the total amount applied, respectively. Around 30% of the total applied amount of thiacloprid was found at the end of the study in all treated mesocosms (around 23% in the water and 7% in the sediment).

Biological Findings:
The biological data showed some minor and major, direct and indirect effects on some groups of organisms, as indicated in the following tables. In these tables, the effects were classified according to the following effect categories suggested in the "Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC" (SANCO 3268/2001 rev.4 (final) of 17 October 2002.

Classification of effects		
1	effect could not be demonstrated	no (statistically significant) effects observed as result of the treatment, and observed differences between treatment and controls show no causal relationship
2	slight effect	effects reported in terms of "slight" or "transient" and/or other similar descriptions, and short-term and/or quantitatively restricted response of sensitive endpoints, and effects only observed at individual samplings
3	pronounced short-term effect	clear response of sensitive endpoints, but total recovery within 8 weeks after the last application, and effects reported as "temporary effects on less sensitive species/endpoints" and/or other similar descriptions, and effects observed at some subsequent sampling instances
4	pronounced effect in short-term study (not relevant in this study)	clear effects (such as strong reductions in densities of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after the (last) application
5	pronounced long-term effect	clear response of sensitive endpoints and recovery time of sensitive endpoints is longer than 8 weeks after the last application, and effects reported as "long-term effects on many sensitive species/endpoints" and/or other similar descriptions, and effects observed at various subsequent samplings.

The NOEAEC (No Observed Ecologically Adverse Effect Concentration) is assumed to be the highest concentration with only effects up to class 3 (short-term effects with recovery within 8 weeks).

Effect classes for the various taxa are summarized in the following tables:

Zooplankton	Test concentration [µg/L]				
	0.5	1.1	2.5	5.5	11.9
Cladocera	2	2	3	3	3
Daphnia magna	1	2	2	3	5
Daphnia longispina / pulex	2	2	3	3	3
Chydorus sphaericus	1	1	1	2	3
Simocephalus, Scapholeberis	1	1	1	+	+
Copepoda	2	2	2	3	3
Copepodits and adults	1	1	1	3	3
Nauplii	2	2	2	3	3
Rotifers		+	+	+	++
Lepadella cf. quadricarinata	1	1	1	1	1
Keratella quadrata	1	1	+	+	+
Polyarthra spec.			+	+	++
Synchaeta spec.		1	1	+	++
Taxa richness	1	1	1	+	+
Diversity	1		+		+
Similarity	1	2	2	3	3
PRCs	1	2	2	3	5
Community-NOEC	**< 0.5**				
Lowest population-NOEC	**< 0.5**				
NOEAEC				**X**	

Insect emergence	Test concentration [µg/L]				
	0.5	1.1	2.5	5.5	11.9
Total emergence	1	1	2	3	3
Chironomidae	1	1	2	3	3
Chironominae	1	1	2	3	3
Chironomus spec.		1	+	+	+
Einfeldia spec.	1	1	1	5	5
Microsectra spec.	1	1	3	5	5
Parachironomus spec.			+	+	++
Tanytarsini	1	1	2	5	5
Orthocladiinae	1	1	1	1	3
Cricotopus spec.	1		1	1	1
Tanypodinae	1	1	2	3	3
Culicidae	1	1	1	+	+
Chaoboridae	1	1	1	1	5
Ephemeroptera (*Cloeon*)		1	1	1	5
Similarity	1	1	2	3	3
PRCs	1	1	2	5	5
Community-NOEC		**X**			
Lowest population-NOEC		**X**			
NOEAEC			**X**		

+ slight or short-term increase, ++ more pronounced long-term increase

Macroinvertebrates in ASS	Test concentration [µg/L]				
	0.5	1.1	2.5	5.5	11.9
Macroinvertebates in total	1	1	1	1	2
Chironomus plumosus	1	1	+	++	3
other Chironomidae	1	1	1	5	5
Tubificidae	1	1	+	+	+
Helobdellla	1	1	1	1	1
Radix ovata	1	1	1	1	+
Gyraulus albus	1	1	1	1	++
Taxa richness	1	1	1	1	1
Diversity	1	1	1	1	1
Similarity	1	1	1	5	5
PRCs	1	1	1	5	5
Community-NOEC			X		
Lowest population-NOEC			X		
NOEAEC			X		

Macroinvertebrates in sediment	Test concentration [µg/L]				
	0.5	1.1	2.5	5.5	11.9
Macroinvertebates in total	1	1	1	1	1
Chironomidae	1	1	1	3	3
Tubificidae	1	1	1	1	1
Gyraulus albus	1	1	1	1	1
Lowest population-NOEC			X		
NOEAEC				X*)	

Asellus aquaticus	Test concentration [µg/L]				
	0.5	1.1	2.5	5.5	11.9
Mesocosms without artificial immigration	2	5	5	5	5
Mesocosms with artificial immigration	2	2	2	2	3
Bioassay	1	2	2	2	3
Lowest population-NOEC	< 0.5				
NOEAEC				X*)	

\+ slight or short-term increase, ++ more pronounced long-term increase

*) NOEAEC set as 5.5 µg/L because 11.9 µg/L was not replicated.

Primary produces	Test concentration [µg/L]				
	0.5	1.1	2.5	5.5	11.9
Total phytoplankton	+	+	+	+	+
Chlorophyceae	1	1	+	+	++
Cryptophyceae	+	+	+	+	+
Diatomeae	1	1	+	+	+
Chlorella minuta	1	1	1	1	1
Chroomonas spec.	1	1	1	1	1
Cryptomonas spec.	+	+	+	+	+
Taxa richness	1	1	1	1	+
Diversity	1	1	1	1	+
Similarity*	1	2	2	2	3
PRCs	1	+	+	++	++
Chlorophyll a in the water	1	+	+	+	+
Periphyton	1	1	1	1	+
Macrophytes	1	1	1	1	1
Total primary production (O2, pH)	1	+	+	+	+
Total primary production (conductivity)	1	1	1	+	+
Community-NOEC	X				
Lowest population-NOEC	< 0.5				
NOEAEC				X*)	

+ slight or short-term increase, ++ more pronounced increase
*) NOEAEC set to 5.5 µg/L due to missing replication at 11.9 µg/L

Within the **zooplankton** community, *Daphnia* species and Cyclopoida - especially nauplii - were identified as the most sensitive zooplankton taxa with statistically significant effects at all test concentrations. With a NOEC of 2.5 µg/L *Chydorus sphaericus* was less sensitive. For other taxa, no consistent negative effects on abundance were found. Some rotifer species and two cladoceran species were promoted with a lowest consistent NOEC of 0.5 µg/L obviously by secondary effects (reduced competition). The similarity indices reflected the toxic effects on the zooplankton with a community NOEC of < 0.5 µg/L, whereas the PRC indicates a NOEC of 0.5 µg/L.

From day 35 on, in most cases no consistent effects on the zooplankton were found up to 5.5 µg/L demonstrating recovery of the zooplankton within 5 weeks. Only for *D. magna* recovery at 11.9 µg/L could not be demonstrated. The rotifer *Synchaeta spec.* was absent in the controls but present in the treated mesocosms until the end of the study. These two taxa showed the highest respectively lowest species weights and thus, they were responsible for the deviation of the PRC of 11.9 µg/L from the controls until the end of the study. Therefore, the NOEAEC for zooplankton in this study is 5.5 µg/L.

The test item affected the **aquatic insects** sampled by **emergence** traps. Consistent effects over at least two sampling dates were observed directly after application for abundant chironomid taxa at concentrations of 2.5 µg/L and higher. Culicidae were the only taxon which seems to benefit indirectly from the test item application at the two highest treatment levels. Thus, the NOEC on the population and community level is 1.1 µg/L. Several taxa showed no or only low emergence until the end of the study in the two highest treatment levels, while no significant consistent effects were found at the end of the study at 2.5 µg/L. Therefore, the NOEAEC for emerged insects is 2.5 µg/L.

Macroinvertebrates were monitored in Artificial Substrate Samplers (ASS) and sediment samples. Direct effects on macroinvertebrates were detected for chironomid only, starting at 5.5 µg/L. Due to the reduction of the abundant taxa, other populations like Tubificidae, *Gyraulus albus* and *Radix ovata*

benefited temporarily, but often not in a dose related way. Thus, the NOEC for Macroinvertebrates is 2.5 µg/L. Because, chironomids showed no clear recovery in the ASS until the end of the study in the two highest treatment levels, the NOEAEC is also 2.5 µg/L.

Asellus aquaticus, a species known to be very sensitive to Deltamethrin, was artificially introduced into the mesocosms before the application. Effects were observed at all treatment levels: At the lowest level, a slight reduction of activity was observed, but population growth was possible again within a few weeks. Due to unlimited growth of the control population, the control levels could not be reached, but the population growth rate was comparable. At higher concentrations, populations became extinct or almost extinct. However, up to 5.5 µg/L possible recolonisation after two weeks was demonstrated by insertion of new organisms into test ponds. At the highest treatment level, survival of immigrants was possible at least after 7 weeks. Laboratory bioassays also showed after eight weeks full survival up to the highest treatment level. Because the mesocosm represents a worst case situation for recovery of animals like *Asellus*, which do not have resting stages (like daphnids) or are not able to immigrate by flying (like insects), the successful artificial immigration and the survival in the bioassays demonstrate the recovery potential in the field by immigration of organisms from adjacent water bodies . Thus, the potential of recovery within eight weeks is shown up to 11.9 µg/L. However, because of the missing replication of this concentration, the NOEAEC for *Asellus* in this study is set to 5.5 µg/L. The test item had no direct effects on the phytoplankton but as an indirect effect some algae species showed a concentration related increase after application because of the reduced grazing of zooplankton organisms. Based on significantly higher abundances of Cryptomonas in all treated mesocosms in the first week after application, the population-NOEC is < 0.5 µg/L. Based on short-term effects on the community level, also indicated by the PRCs, the Community-NOEC is set to 0.5 µg/L. At all treatment levels, the algae blooms occurred only a few weeks. Because the highest treatment level was not replicated, the NOEAEC is set to 5.5 µg/L. No statistically significant effects were found for macrophytes (*Elodea canadensis*) and periphyton (filamentous algae), but a slightly increased growth was observed at 2.5 µg/L and higher for periphyton and at 11.9 µg/L for macrophytes. Chlorophyll concentrations and indicators of total primary production (oxygen concentrations, pH, and conductivity) confirm the indirect growth of the phytoplankton.

Conclusion

The accompanying chemical analysis demonstrates a steady and fast decline of Deltamethrin in the mesocosm water with a mean DT_{50} of 2.1 days and a mean of 2.5 days for the whole test system (water plus sediment). In the sediment of the two lowest test concentrations Deltamethrin was not found. The results of the higher test concentrations show a slight increase of the amount of the test substance in the sediment for about the first six weeks after application and a slow but constant decrease thereafter. Thiacloprid was more persistent with a DT_{50} of about 43 days for the water and 48 days for the whole system. Thiacloprid was found in the sediment of all treated mesocosms and showed a slower increase in the sediment during the study period over the first seven weeks with a decrease until the end. Some crustaceans were identified as the most sensitive taxa with effects even at 0.5 µg/L. For Daphnids and cyclopodite copepods a recovery within a few weeks could be demonstrated up to 5.5 µg/L. *Asellus aquaticus* showed a normal population growth a few weeks after application at 0.5 µg/L. At the higher treatment levels, the introduction of new *Asellus* demonstrated the potential for recolonisation at concentrations up to 11.9 µg/L. The bioassay findings confirm that water and food samples taken from the mesocosms a few weeks after application did not have any negative effects on *Asellus aquaticus* survival. Therefore, a fast recovery is concluded even for the most sensitive species up to the highest test concentration. The NOEAEC for the crustaceans was set to 5.5 µg/L because of the missing replication of 11.9 µg/L. Chironomid species were identified as the most abundant insects and macroinvertebrates in general, according to the emergence, ASS and sediment data sets. Effects on these

midges were observed at initial concentrations of 2.5 µg/L and higher. Full recovery could only be demonstrated for all species at 2.5 µg/L. Thus the NOEAEC for these midges is 2.5 µg/L.
The direct effects on the daphnids, copepods, *Asellus* and chironomids caused indirect effects on the phytoplankton (reduced predation) and on competitors like rotifers, Culicidae, and maybe snails and Tubificidae (reduced competition). However, in most cases these indirect effects were only temporarily and restricted to higher treatment levels.

Taking into account all the findings summarized above, an overall NOEAEC (No Observed Ecological Adverse Effect Concentration) of 2.5 µg/L Thiacloprid & Deltamethrin OD 110 can be derived from this study. This test item concentration corresponds to a nominal peak concentration of 242 ng thiacloprid/L and 23 ng deltamethrin/L, respectively.

*****

Report:	**KCP 10.2.3/07; [redacted]; 2007**
Title:	Deltamethrin EW 15 G: Acute and chronic Effects to Different Life Stages of the Isopod *Asellus aquaticus* L. in a Natural Water-Sediment System
Document No:	M-291885-02-1 (P1MA)
Guidelines:	-
GLP:	Yes

Material and methods:
Test item: Deltamethrin EW 15G, purity: 1.5% w/w deltamethrin, batch no.: OP240778, specification no. 102000003191
In this laboratory test the ecological effects of Deltamethrin EW 15 on the aquatic invertebrate *Asellus aquaticus* were studied. Different life stages of *Asellus aquaticus* were tested in two separate approaches, one with juveniles and one with adults. The animals were collected in natural ditches in [redacted] (The Netherlands), from where the natural water and sediment was collected and transferred to the test facility.
The following biological parameters were monitored: Mortality, sublethal effects (such as reduced activity) and the time taken for recovery if effects occur.
The endpoints of the study were: LC_{50}, NOEC, mortality and sublethal effects.
Nominal test concentrations were 0, 1.0, 2.2, 4.8, 10.6, 23.4 and 51.5 ng a.s./L (number of replicates: three for each life stage). Ten test organisms of each life stage were introduced randomly into corresponding test vessels 8 days before application. Exposure period was 21 days; the system has been equilibrated and conditioned for approximately 12 days before the application of the test item.
The test conditions were: temperature: 18.3 – 20.3 °C, light regime: 16 light:8 dark, light intensity: 100 – 500 lux, aeration of test chambers: gentle aeration, feeding: 10 leaves of pre-conditioned *Populus canadensis* per replicate; the leaves were introduced into the test vessels 11 days before the test item application.
For each test item concentration three additional replicates without test species were prepared for analytical purposes. These replicates were treated in the same way as the test systems with test species. Samples for analytical purposes were taken form the overlying water column of all additional test vessels 2 – 4 hours after application and at each observation point for each concentration level. Sediment samples were taken for the three highest concentrations on day 7, 14 and 21 of the test period. The chemical analysis was performed by Bayer CropScience AG.

Findings:

Analytical findings: The analysed concentrations of the stock solutions confirm the nominal test concentrations. After application the concentration of deltamethrin in test water decreased rapidly. The total recovery of all introduced individuals was not possible at the interim sampling dates (e.g. due to turbidity in the test vessels). At the end of the test period (21 days after application) a final assessment was performed by emptying the test vessels and searching through the sediment for surviving test organism. Therefore, the final evaluation is the most relevant one.

Biological findings (survival): The survival of *Asellus aquaticus* after 21-day exposure to deltamethrin in a static water-sediment system is summarized in the table below. Survival of both, juvenile and adult organisms was significantly reduced in the highest test concentrations. No difference in sensitivity of the two life stages was observed.

Percentage of survival as mean of 3 replicates at day 21

Nominal deltamethrin concentration	Survival at day 21 [%]	
	Juveniles	Adults
Control	76.7	83.3
1.0 ng a.s./L	90.0	93.3
2.2 ng a.s./L	93.3	80.0
4.8 ng a.s./L	73.3	83.3
10.6 ng a.s./L	66.7	93.3
23.4 ng a.s./L	86.7	76.7
51.5 ng a.s./L	26.7	30.0

Other observations:

In the test vessels with initially introduced adults of *Asellus aquaticus*, newborns were observed for the first time 4 days after application up to a concentration of 23.4 ng a.s./L. In the highest concentration (51.5 ng a.s./L) the first newborns appeared only in one replicate 14 days after application.

LC_{50}, LOEC and NOEC:

The following LC_x-, NOEC and LOEC values were calculated for juveniles and adults of *Asellus aquaticus*:

Estimated LCx mortality LOEC and NOEC in ng a.s./L for adult *Asellus aquaticus* based on statistical evaluation of biological results and nominal (initial) concentrations for day 21. Control mortality was compensated using Abbott's formula

	0 – 21 d		
	Endpoint [ng a.s./L]	**Lower 95% confidence interval**	**Upper 95% confidence interval**
LC_{10}	24.7	14.7	41.7
LC_{20}	30.1	20.4	44.5
LC_{50}	43.9	34.8	55.3
LOEC	51.5		
NOEC	**23.4**		

Estimated LCx mortality LOEC and NOEC in ng a.s./L for juvenile *Asellus aquaticus* based on statistical evaluation of biological results and nominal (initital) concentrations for day 21. Control mortality was compensated using Abbott's formula

	0 – 21 d		
	Endpoint [ng a.s./L]	**Lower 95% confidence interval**	**Upper 95% confidence interval**
LC_{10}	28.2	19.6	40.5
LC_{20}	33.1	25.0	42.8
LC_{50}	44.8	36.7	54.8
LOEC	51.5		
NOEC	**23.4**		

Conclusion:
In a static water-sediment laboratory system, the IC_{50}-values observed 21 days after application of deltamethrin was 43.9 ng a.s./L for adults and 44.8 ng a.s./L for juveniles of *Asellus aquaticus* based on nominal concentrations. The NOEC was 23.4 ng a.s./L for juveniles and adults. It can be concluded that the sensitivity of of juvenile *A. aquaticus* to deltamethrin is equal to the sensitivity of adult organisms.

*****

The test concentrations of the study summarized above were evaluated in the following reports:

Report:	**KCP 10.2.3/08; [redacted]; 2007**
Title:	Analysis of deltamethrin concentrations in water samples of LCT study no. P1MA
Document No:	M-291848-01-1 (MR-07/295)
Guidelines:	-
GLP:	Yes

Report:	**KIIIA 10.2.3/09, [redacted]; 2007b**
Title:	Modification M001 of analytical method 00886 for the determination of total residues of deltamethrin (AE F032640) in surface water by HPLC-MS/MS
Document No:	M-291746-01-1
Guidelines:	Does not apply
GLP:	no

Summary:
This method M00886/M001 describes the determination of deltamethrin in test water from aquatic toxicity tests by HPLC-MS/MS and provides validation data for test water using Multiple Reaction Monitoring (MRM).
Water samples are analysed after addition of acetonitrile and internal standard solution by direct injection into an HPLC-MS/MS instrument using electrospray ionization in the positive mode.
The mass spectrometric detector showed linear response in the concentration range of 2 ng/L to 100 ng/L with a correlation coefficient of 0.9995 (1/x weighted).
Repeatability testing of MS/MS detection of deltamethrin in test water samples fortified at concentrations of 2 ng/L and 10 ng/L yielded a relative standard deviation (RSD) for the peak area ratio of 2.4% and 3.3%. The RSD for the retention time was ≤ 0.2% for both fortification levels.
The limit of quantitation (LOQ) for deltamethrin is 2 ng/L.
The limit of detection was 0.5 x LOQ.

Report:	**KCP 10.2.3/10, [redacted] 2007**
Title:	Analysis of deltamethrin concentrations in sediment samples of ECT study no. P1MA
Document No:	M-291818-01-1 (MR-07/297)
Guidelines:	-
GLP:	Yes

*****

Report:	**KCP 10.2.3/11, [redacted]; 2007**
Title:	Brief Summary of Methods and first Results (non-GLP) of the Cancelled Microcosm Study on Chronic Effects of deltamethrin EW 15 G on Population Dynamics of the Isopod *Asellus aquaticus* L. in a Natural Water-Sediment System
Document No:	M-291879-01-1
Guidelines:	No Guideline available
GLP:	No

The study was aimed at determining the chronic effects (such as population dynamics and potential recovery) of deltamethrin towards a population of different age (size) classes of *Asellus aquaticus* in a water-sediment system under realistic spray exposure conditions. However, since the life stage study (see above; M-291885-02-1) provided reliable results and the interpretation of results from a population study with different age classes is difficult, the microcosm study was terminated five weeks after study implementation. Nevertheless, the results obtained confirm the results of the life stage study:

- Survival of juvenile and adult *A. aquaticus* was not affected after 35 d of exposure to deltamethrin in a static water-sediment-system up to a nominal peak concentration of 51.5 ng a.s./L
- No difference in sensitivity between juvenile and adult organisms was observed.

*****

Report:	**KCP 10.2.3/12, [redacted]; 2007**
Title:	Drift of the freshwater isopod *Asellus aquaticus* in a stream in an agricultural landscape – a case study
Document No:	M-291925-01-1
Guidelines:	No Guideline available
GLP:	No

Objective:
A field study was conducted to examine the drift behaviour of the freshwater isopod *Asellus aquaticus*.

Material and methods:
Field studies were conducted in [redacted], a third order stream (width: about 1.2 m, depth: about 20 cm, flow velocity: between 0.01 and 0.2 m/s) discharging agricultural land (mainly vineyards) close to [redacted] South Western Germany. The stream itself is considered to belong to the category "ditch" when applying a scheme on FOCUS scenarios.
Population density: Population densities of *A. aquaticus* were determined between June and August 2007 with a Surber sampler (are: 0.062m²; 1 mm mesh). Five independent samples were taken each month over a stream length of 500 m.

Drift rate determination: The drift of isopods was measured with continuously operating bottom drift nets (opening 10 x 4 cm; net length 200 cm; mesh size 1 mm) at a total of 10 sites. Each sampling interval lasted for 24 hours. Additional drift rates were measured in the blocking experiments described below.

Drift distance (blocking experiments): These experiments were designed to determine the effect of blocking the total drift upon drift rates of *A. aquaticus* at points located downstream of the mesh barrier used as a block. If the occurrence of organisms accumulating in the mesh barrier was the result of only random activity in the immediate vicinity of the mesh barrier, and the individuals found there were not in the process of moving downstream, then drift rates in locations downstream of the block would be unaffected. On the other hand, if the isopods stopped at the block had been moving downstream and were about to contribute to the drift further downstream, then drift rates down from the block would be correspondingly reduced. Two blocking experiments were conducted in July and August 2007. The block consisted of a coarse metal mesh (mesh size: 1 cm) to retain larger detritus followed by a fine wire mesh (mesh size: 1 mm) positioned downstream. Both blocking nets covered the complete cross section of the water body to make sure that macroinvertebrates were not able to pass the net from either side.
The drift of the isopods was measured with continuously operating bottom drift nets (see above) at a total of seven sites, situated downstream at distances of 2, 4, 6, 8, 10, 12 and 14 m (1st experiment) and 2, 4, 6, 10, 15, 25 and 50 m (2nd experiment), respectively. Drift measurements started 1 h after installation of the mesh barrier in two (1st experiment) and four (2nd experiment) 24-hour intervals. Flow velocities were measured about 15 cm upstream of the drift net openings.

Findings:
Samples taken in [redacted] resulted in a mean population density of 2223±1040 ind/m² (n = 15), which represents a moderate to high density for this species.
Drift rates ranged from 225 to 1918 individuals/24 h (mean drift rate: 675 ± 467 ind/24 h; n=45). Measured flow velocities varied between 0.01 and 0.2 m/s at the different sampling sites and intervals. Based on all drift data and corresponding flow velocity measurements, there is no correlation between the drift rate and the flow velocity at a given site and time. The data show a pronounced activity and movement of *A. aquaticus* over the full range of observed flow velocities.
The results from the 1st drift distance experiment suggest that the effect of the mesh barriers is visible at 14 m (i.e. maximum distance examined), i.e. the relevant drift distances do cover stretches of this length. The 2nd experiment indicated that distances of up to 25 m seem to be relevant drift distances for *A. aquaticus*.

Conclusion:
Even if it was not conclusively possible in this study to distinguish between active or passive components or between drift and locomotion, the data suggest a rather high spatial dynamic for the isopod species *A. aquaticus*.

*****

Report:	**KCP 10.2.3/13, [redacted]; 2007**
Title:	Biology and distribution of selected waterlice and freshwater shrimps of Central Europe – a literature review
Document No:	M-291865-01-1
Guidelines:	No Guideline available
GLP:	No

Objective:
The aim of this report was to accumulate data on the biology and distribution of selected waterlice and freshwater shrimps of Central Europe. It summarizes information on reproduction and life cycle, the preferred habitat of the different species, but also to describe the variation of occurrence, the geographic distribution pattern in Central Europe, food preferences and interactions with other species focusing on other species in the same group.

Material and methods:
In total over 100 primary sources (publications) were reviewed for relevant information on the waterlice *Asellus aquaticus*, *A. coxalis* and *A. meridianus* and the amphipods *Gammarus pulex*, *G. fossarum*, *G. roeselii* and *G. fasciatus*. The collection of this information was in most cases not of a quantitative manner and not carried out with a standardized methodology.

Findings:
The biology of the following species was addressed in this paper: the isopods *Asellus aquaticus*, *A. coxalis* and *A. meridianus*, and the amphipods *Gammarus pulex*, *G. fossarum*, *G. roeselii* and *G. fasciatus*.
Gammarus fasciatus is a North American species mainly inhabiting upstream brooks with clear and fast flowing water, and was not considered relevant for European agricultural areas.
Due to its robustness agains pollution, low oxygen and low pH, *Gammarus pulex* was identified as the dominant *Gammarus* species in areas with these environmental constraints, resulting in high abundances especially in lowland brooks and ditches in the agricultural landscape.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

Table 10.3.1- 1: Acute toxicity of deltamethrin (tech.) to honey bees

Test substance	Test species/study design	Endpoint	Reference
Deltamethrin, tech.	Honey bee, 48 h	LD_{50} – contact ≈ 0.0015 µg a.s./bee	[redacted] (1991) M-149380-01-1 List of Endpoints (EU review report, 2002)
Deltamethrin, tech.	Honey bee	LD_{50} – oral 0.079 µg a.s./bee LD_{50} – contact 0.051 µg a.s./bee	[redacted] (1978) M-098831-01-1 List of Endpoints (EU review report, 2002)
Deltamethrin, tech.	Honey bee	LD_{50} – oral 0.079 µg a.s./bee LD_{50} – contact 0.047 µg a.s./bee	Anonymous (1977) M-150494-01-2 KCA 8.3.1.1/01
Deltamethrin, tech.	Honey bee, 72 h	LD_{50} – oral 0.19 µg a.s./bee LD_{50} – contact 0.11 µg a.s./bee	[redacted] (2013) 73581035 M-144971-01-1 KCA 8.3.1.1/02
Deltamethrin, technical concentrate	Honey bee, 72 h	LD_{50} – oral 0.023 µg a.s./bee	[redacted] (1996) CW 94/084 M-140579-01-1 KCA 8.3.1.1/03
Deltamethrin, technical concentrate	Honey bee, 72 h	LD_{50} – contact 0.012 µg a.s./bee	[redacted] (1996) CW 94/083 M-149608-01-1 KCA 8.3.1.1.2/02

Toxicity of deltamethrin to bumble bees in the laboratory

There is currently no harmonized and ring tested test guideline available in Europe to assess the acute toxicity to bumble bees; this is particularly true for the oral route of exposure, as bumble bees do not share their food through trophallaxis. For the determination of the contact toxicity of deltamethrin to bumble bees, the honey bee test method has been adopted, wherever possible.

Table 10.3.1- 2: Acute toxicity of deltamethrin (tech.) to bumble bees

Test substance	Test species/study design	Endpoint	Reference
Deltamethrin, tech.	Bumble bee, 96 h	LD_{50} – contact 36.0 µg a.s./bee	[redacted] (2014) S13-04467 M-477381-01-1 KCA 8.3.1.1.2/03

Table 10.3.1- 3: Acute toxicity of formulated deltamethrin to honey bees

Test substance	Test species/study design	Endpoint	Reference
Deltamethrin, EW 15	Honey bee, 72 h	LD_{50} – oral **0.067 µg a.s./bee** LD_{50} – contact **0.078 µg a.s./bee**	[redacted] (2003) 031048047 M-103549-01-1 KCP 10.3.1.1.1/01
Deltamethrin, EW 15	Honey bee, 72 h	LD_{50} – oral 0.294 µg a.s./bee	[redacted] (2000) CW 00/027 M-199244-01-1 KCP 10.3.1.1.1/02
Deltamethrin, EW 15	Honey bee, 72 h	LD_{50} – contact 0.013 µg a.s./bee	[redacted] (2000) CW 00/032 M-198509-01-1 KCP 10.3.1.1.2/01
Deltamethrin, EW 50	Honey bee, 48 h	LD_{50} – oral 0.79 µg a.s./bee LD_{50} – contact 0.12 µg a.s./bee	[redacted] (2006) 05 10 48 103 M-270468-01-1 KCP 10.3.1.1.1/03
Deltamethrin, EC 15	Honey bee, 72 h	LD_{50} – oral 0.415 µg a.s./bee	[redacted] (2000) CW 00/026 M-199148-01-1 KCP 10.3.1.1.1/04
Deltamethrin, EC 15	Honey bee, 72 h	LD_{50} – contact 0.025 µg a.s./bee	[redacted] (2000) CW 00/030 M-198885-01-1 KCP 10.3.1.1.2/02
Deltamethrin, EC 25	Honey bee, 48 h	LD_{50} – oral 0.28 µg a.s./bee 0.010 µg LD_{50} – contact a.s./bee 0.108 µg a.s./bee	[redacted] (1987) LEA/I/87-005 M-118451-01-1 KCP 10.3.1.1.1/05
Deltamethrin, EC 25	Honey bee, 48/72 h	LD_{50} – oral 0.143 µg a.s./bee LD_{50} – contact 0.108 µg a.s./bee	[redacted] (2008) 33041035 M-309900-01-1 KCP 10.3.1.1.1/06
Deltamethrin, EC 100	Honey bee, 72 h	LD_{50} – oral 0.266 µg a.s./bee	[redacted] (2000) CW 00/028 M-199150-01-1 KCP 10.3.1.1.1/07
Deltamethrin, EC 100	Honey bee, 72 h	LD_{50} – contact 0.028 µg a.s./bee	[redacted] (2000) CW 00/031 M-198786-01-1 KCP 10.3.1.1.2/03
Deltamethrin, EG 06	Honey bee, 72 h	LD_{50} – oral 0.182 µg a.s./bee	[redacted] (1995) CW94/067E M-134668-01-1 KCP 10.3.1.1.1/08
Deltamethrin, EG 06	Honey bee, 72 h	LD_{50} – contact 0.064 µg a.s./bee	[redacted] (1995) CW94/030E M-133791-01-1 KCP 10.3.1.1.2/04

Bold values: Endpoints considered relevant for risk assessment

Foliage residual toxicity of deltamethrin to honey bees in the laboratory

There is currently no harmonized and ring tested European test guideline to assess the residual toxicity of pesticides to honey bees. However, there is an US EPA test guideline established which investigates the residual toxicity of aged foliar spray deposits on honey bees under laboratory conditions.

Table 10.3.1- 4: Foliage residual toxicity of deltamethrin to honey bees in the laboratory

Test substance	Results	Reference
Deltamethrin EC 25	Exposure of honey bees to foliar residues (following foliar application of 22.4 g a.s./ha) aged over 2, 8 and 24 hours under field condition resulted in 31%, 21% and 5% mortality rates. With the 24h old residues, there was no statistical significant difference in mortality as compared to the untreated control. The RT_{25} at a rate of 22.4 g a.s./ha was less than 8 hours. For the rate of 22.4 g a.s./ha, a minimal hazard for honey bees was concluded when honey bees are not actively foraging during application (e.g. evening application).	[redacted] (1992) US88303 M-166394-01-1

RT_{25} = residual time at which mortality rate is less than 25%

Chronic toxicity of deltamethrin to adult honey bees

There is currently no harmonised and ring tested European test guideline to assess the chronic risk to adult honey bees. To address this endpoint, available guidance as well as recently developed working group experience was applied to feed honey bees exclusively and *ad libitum* with treated 50% (w/v) sugar solutions for a period of 10 consecutive days. The respective feeding study was conducted with formulated deltamethrin (Deltamethrin EW 15) due to the very low water solubility of the technical grade material.

Table 10.3.1- 5: Chronic toxicity of deltamethrin to adult honey bees

Test substance	Test species/study design	Endpoint	Reference
Deltamethrin EW 15	10 d chronic adult feeding study	LC_{50} 15.1 mg a.s./kg LDD_{50} 0.53 µg a.s./bee/day	[redacted] (2014) S13-00151 M-477250-01-1 KCA 8.3.1.2/01

$LDD_{(50)}$: Lethal Dietary Dose$_{(50)}$

Effects of deltamethrin on honey bees and honey bee colonies under forced exposure conditions (i.e. in the semi-field, under confinement), when applied during full bloom to highly bee attractive crops during honey bees actively foraging

Several studies under forced exposure conditions have been conducted to examine the risk posed by foliar applications of formulated deltamethrin to honey bees. In order to maximize exposure of honey bees to deltamethrin residues, the product was applied to the highly bee attractive surrogate crop *Phacelia tanacetifolia* during honey bees actively foraging. These studies followed either the provisions of the respective current version of French CEB-guidelines or the provisions of the respective current version of the EPPO 170 guideline; one study additionally complied with the provisions of the OECD Guidance Document 75 (detailed photographic brood assessment). The investigated application rates covered application rates between 5 - 12.5 g a.s./ha.

Table 10.3.1- 6: Cage and tunnel studies (forced exposure) with honey bees (*Apis mellifera* sp.) actively foraging in flowering, highly bee-attractive crops

Test substance	Study design	Results	Reference
Deltamethrin EW 15	7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Slight increase in mortality, no or very transient effect on foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) S01AVB.877VO44 M-204260-01-1 KCP 10.3.1.5/01
Deltamethrin EW 15	7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Slight-moderate, transient increase in mortality, slight-moderate, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2005) S05BAB.DELVO16 M-272845-01-1 KCP 10.3.1.5/02
Deltamethrin EW 15	7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Moderate, transient increase in mortality, slight, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2000) 2000-23.2 M-198218-01-1 KCP 10.3.1.5/03
Deltamethrin EW 15	7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Slight increase in mortality, slight decrease in foraging activity, no adverse effects on brood- and colony development (digital imaging as well as overall brood assessment)	[redacted] (2014) S12-00441 M-477316-01-1 KCP 10.3.1.5/04
Deltamethrin EW 50	7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Transient increase in mortality, (transient) decrease in foraging activity, no adverse effects on brood- and colony development	[redacted], S (2006) 29011037 M-274120-01-1 KCP 10.3.1.5/05
Deltamethrin EC 15	7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Transient increase in mortality, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) 20001132/01-BZEU M-200402-01-1 KCP 10.3.1.5/06
Deltamethrin EC 25 & EW 15	5 & 7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Slight, transient increase in mortality, slight, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) 36-2001 M-205048-01-1 KCP 10.3.1.5/07
Deltamethrin EC 25 & EC 100	5 & 7.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Slight, transient increase in mortality, slight, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) 35-2001 M-205046-01-1 KCP 10.3.1.5/08
Deltamethrin EC 25 & EG 06	12.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Moderate, transient increase in mortality, slight-moderate, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2000) 991048103 M-197723-01-1 KCP 10.3.1.5/09
Deltamethrin EC 25 & EG 06	12.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Moderate, transient increase in mortality, slight, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2000) 99379/01-BZEU M-195280-01-1 KCP 10.3.1.5/10
Deltamethrin EC 25 & EG 06	12.5 g a.s./ha in full-flowering *Phacelia*, bees actively foraging	Slight increase in mortality, no or very transient effect on foraging activity, no adverse effects on brood- and colony development	[redacted] (1999) 999 M-195036-01-1 KCP 10.3.1.5/11

Effects of deltamethrin on honey bees and honey bee colonies under forced exposure conditions (i.e. in the semi-field, under confinement), considering foliar application to cereals in a simulated honey dew scenario during honey bees actively foraging

Several semi-studies under forced exposure conditions have been conducted to examine the risk posed by foliar applications of formulated deltamethrin to cereal crops which may attract honey bees due to honeydew formation at high aphid infestation levels. The studies followed the provisions of the respective current version of French CEB-guidelines under consideration of the provisions of the respective current version of the EPPO 170 guideline. The investigated application rate was 6.25 g a.s./ha.

Table 10.3.1- 7: Cage and tunnel studies (forced exposure) with honey bees (*Apis mellifera* sp.) actively foraging on cereal crop which had been treated with sugar solution prior to application of the test item to simulate a honey dew scenario

Test substance	Study design	Results	Reference
Deltamethrin EW 15	6.25 g a.s./ha on sugar-treated cereals, bees actively foraging	Slight increase in mortality, no effect on foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) S01AVB879VO45 M-203985-01-1 KCP 10.3.1.5/12
Deltamethrin EW 15	6.25 g a.s./ha on sugar-treated cereals, bees actively foraging	No increase in mortality, no or limited effect on foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) S00AGB3264VO56 M-205201-01-1 KCP 10.3.1.5/13
Deltamethrin EW 15	6.25 g a.s./ha on sugar-treated cereals, bees actively foraging	Slight increase in mortality, slight and transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2005) [redacted]/AM039 M-262484-01-1 KCP 10.3.1.5/14
Deltamethrin EC 100	6.25 g a.s./ha on sugar-treated cereals, bees actively foraging	Slight, transient increase in mortality, slight, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) 33-2001 M-201580-01-1 KCP 10.3.1.5/15
Deltamethrin EC 100	6.25 g a.s./ha on sugar-treated cereals, bees actively foraging	Slight, transient increase in mortality, slight, transient decrease in foraging activity, no adverse effects on brood- and colony development	[redacted] (2006) 87-2005 M-268997-01-1 KCP 10.3.1.5/16
Deltamethrin EC 100	6.25 g a.s./ha on sugar-treated cereals, bees actively foraging	No increase in mortality, limited effect on foraging activity, no adverse effects on brood- and colony development	[redacted] (2001) S00AGB3264VO54 M-205203-01-1 KCP 10.3.1.5/17

Effects of deltamethrin on bumble bees under forced exposure conditions (i.e. in the semi-field, under confinement), during bumble bees actively foraging

There is currently no harmonized and ring tested European test guideline available to assess risks posed by pesticides to bumble bees. To address this endpoint, available honey bee guidance as well experience from greenhouse pollination experiments has been adopted, wherever possible. On the basis of the French CEB-guidelines, bumble bees were exposed to a foliar treatment of formulated deltamethrin, applied to the highly bee attractive surrogate crop *Phacelia tanacetifolia* during bumble bees actively foraging on the crop. The investigated application rate was 12.5 g a.s./ha.

Table 10.3.1- 8: Cage and tunnel studies (forced exposure) with bumble bees (*Bombus terrestris*) actively foraging in a flowering, highly bee-attractive crop

Test substance	Study design	Results	Reference
Deltamethrin EW 15	12.5 g a.s./ha in full-flowering *Phacelia*, bumble bees actively foraging	No effects on mortality, slight, transient decrease in foraging activity	[redacted] (2000) 2000-24.1 M-200040-01-1 KCP 10.3.1.5/18
Deltamethrin EG 06	12.5 g a.s./ha in full-flowering *Phacelia*, bumble bees actively foraging	No effects on mortality, slight, transient decrease in foraging activity	[redacted] (2000) 2000-24.3 M-200043-01-1 KCP 10.3.1.5/19

Investigation of side-effects of formulated deltamethrin on honey bees and honey bee colonies in non-GLP semi-field and field experiments, conducted in the late 1970's and early 1980's

In the context of the early development of deltamethrin for commercial use, effects on honey bees and honey bee colonies were intensively examined in non-GLP (semi-)field studies which provide useful complementary information for the risk characterization of deltamethrin. To facilitate the reading and evaluation of all these data, summary documents were prepared which outline the applied experimental designs and the corresponding findings.

Table 10.3.1- 9: Non-GLP trials during the early development of deltamethrin, to examine the risk posed by foliar treatments of formulated deltamethrin to honey bees and honey bee colonies (*Apis mellifera* sp.), during honey bees actively foraging on bee-attractive crops or on sugar-treated cereals

Test substance	Test system: flowering crop	Results	Reference
Various deltamethrin formulations	Non-GLP field studies, conducted in the late 1970's and early 1980's, exposing honey bees and honey bee colonies to foliar deltamethrin treatments in bee-attractive, flowering crops, during honey bees actively foraging	Application rates of ≤12.5 g a.s./ha did not result in elevated mortality when applied during honey bees actively foraging on bee-attractive crops during bloom. An application rate of 17.5 g a.s./ha during bee flight caused only a very slightly increased mortality, application rates of 21.2 and 25 g a.s./ha during bee flight are about the threshold rates for mortality. At an application rate of 35 g a.s./ha during bee flight, a substantially increased mortality was recorded. Up to and including 17.5 a.s./ha, there was a transient depression of foraging activity (repellent effect). At application rates >17.5 g a.s./ha, the foraging activity was found to be less depressed. Where examined, brood development, honey production as well as overwintering performance of exposed hives was not impacted. Also, only very low or no deltamethrin residues were found in analysed honey and/or pollen samples.	[redacted] (1983; summary document) M-151220-01-1 KCP 10.3.1.6/07 [redacted] (1987; summary document) M-151219-01-1 KCP 10.3.1.6/09 [redacted] (1992; summary document) FR0792 M-149734-01-1 [redacted] (1993; summary document) M-151216-01-1 KCP 10.3.1.6/08 [redacted] (1978) FR7837; 78/Insect. No. 37 M-124929-01-2 [redacted] (1979) FR7935H ; 79/Insect. No. 35-H M-124930-01-1 [redacted] (1980), FR8039; 80/Insect. No. 39/A M-124984-01-1 [redacted] (1981), FR8138H; 81/Insect. No. 38/A M-149768-01-2 [redacted]; [redacted] (1982) FR8239; 82/Insect. No. 39 M-149774-01-2 KCP 10.3.1.6/10

Deltamethrin EC 25	Non-GLP tunnel studies, conducted in the mid 1980's in sugar-treated cereals (simulated, honey dew scenario)	At application rates of 6.25 and 12.5 g a.s./ha, mortality was not increased when the product was applied to sugar-treated cereal crops during honey bees actively foraging. There was a slight and transient decrease of foraging activity. Where examined, no effects on brood development were recorded.	[redacted] (1983) FR832H; 83/ Insect. No. 2-H M-149770-01-2 [redacted] (1984) FR8437; 84/Insect. No. M-149771-01-2

Effects of deltamethrin on honey bees and honey bee colonies under field conditions

Several field studies have been conducted with formulated deltamethrin using highly bee attractive crops and following the provisions of the respectively current version of the EPPO 170 guideline. In some of these studies, further endpoints beyond to the standard assessments were examined, e.g. homing behaviour, bee health parameters (bee diseases, viruses, parasites), overwintering performance and effects of repeated applications.

Table 10.3.1- 10: Field studies with honey bees (*Apis mellifera* sp.) actively foraging in flowering, highly bee-attractive crops

Deltamethrin EC 25	7.5 g a.s./ha in full-flowering *Phacelia*; bees actively foraging	No adverse effects on mortality, flight intensity, behaviour, brood- and colony development	[redacted] (2007) 2006\298/G1-BFEU M-286584-01-1 KCP 10.3.1.6/01
Deltamethrin EC 25	7.5 g a.s./ha in full-flowering oil-seed rape; bees actively foraging; three separate locations, including a detailed homing- behaviour assessment	No adverse effects on mortality, flight intensity, behaviour, brood- and colony development. No impact on homing behaviour.	[redacted] (2007) 20071100/G1-BFEU M-295800-01-1 KCP 10.3.1.6/02
Deltamethrin EC 25	17.5 g a.s./ha in full-flowering *Phacelia*; **evening application, after bee flight**	No adverse effects on mortality, flight intensity, behaviour, brood- and colony development	[redacted] (2009) S09-00073 M-358267-01-1 KCP 10.3.1.6/03
Deltamethrin EC 25	7.5 g a.s./ha in full-flowering *Phacelia*; bees actively foraging	Slight transient increase in mortality, no adverse effects on flight intensity, brood- and colony development	[redacted] (1998) 98299/01-BFEU M-185038-01-1
Deltamethrin EG 06	7.5 g a.s./ha in full-flowering *Phacelia*; bees actively foraging	No adverse effects on mortality, flight intensity, behaviour, brood- and colony development	[redacted] (1998) 98300/01-BFEU M-184784-01-1 KCP 10.3.1.6/04
Deltamethrin EW 15	2 x 12.5 g a.s./ha in full-flowering *Phacelia*; bees actively foraging; long-term bee-health assessments, including over-wintering	No acute, short-term or long-term adverse effects on mortality, flight intensity, colony strength, colony health and vitality, brood & food development and overwintering performance in the exposed colonies	[redacted] (2013) S10-3820 M-452717-01-1 KCP 10.3.1.6/05
Deltamethrin EW 15	2 x 12.5 g a.s./ha in full-flowering *Phacelia*; bees actively foraging; long-term bee-health assessments, including over-wintering	No acute, short-term or long-term adverse effects on mortality, flight intensity, colony strength, colony health and vitality, brood & food development and overwintering performance in the exposed colonies	[redacted] (2013) S10-3824 M-452723-01-1 KCP 10.3.1.6/06

Tier 1 Risk Assessment: Hazard Quotients

A Hazard Quotient (Q_H) approach has been defined by the EPPO risk assessment scheme to identify use patterns which pose a negligible acute risk to honey bees. The Q_H is determined by calculating the ratio between the application rate (expressed in g a.s./ha) and the lowest laboratory contact and oral LD_{50} (expressed in µg a.s./bee).

Q_{HO} and Q_{HC} resp. = Application rate [g a.s./ha] / LD_{50} oral or LD_{50} contact [µg a.s./bee]

Q_H values can be calculated using data from the studies performed with either the active substance or with the formulated product. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honey bees.

For deltamethrin, the calculation of hazard quotients is based on the product Deltamethrin EW 15, which is both, the actual product of the review process and a product which can be considered to be a representative product for formulated deltamethrin.

Table 10.3.1- 11: Hazard quotients for honey bees – oral exposure

Crop	Exposure route	LD_{50} [µg a.s./bee]	Application rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger value	Refined risk assessment required?
Deltamethrin EW 15						
Sugarbeet	oral	0.067	7.5	112	50	**Yes**
Cauliflower			7.5	112		
Wheat			6.25	93		

Table 10.3.1- 12: Hazard quotients for honey bees – contact exposure

Crop	Exposure route	LD_{50} [µg a.s./bee]	Application rate [g a.s./ha]	Hazard quotient Q_{HO}	Trigger value	Refined risk assessment required?
Deltamethrin EW 15						
Sugarbeet	contact	0.078	7.5	96	50	**Yes**
Cauliflower			7.5	96		
Wheat			6.25	80		

The hazard quotients for oral and contact exposure exceed the trigger value for *a priori* non-critical product uses. Therefore, a more refined risk assessment is required, which is presented below.

Refined Assessment of Risks Posed by Deltamthrin to Honey Bees

The deltamethrin use pattern comprises the rate of 7.5 g a.s./ha in sugar beet and cauliflower, considering one or two applications from BBCH 10 - 49. The use in cereal crops comprises two applications of 6.25 g a.s./ha from BBCH 10 - 83.

In commercial sugar beet production, sugar beets are harvested well in advance of flowering (i.e. generally at about BBCH 49: “beet root has reached harvestable size”). Therefore, bees are not attracted to the crop due to the lack of forage, i.e. up to and including BBCH 49, the vegetative crop

stages do not offer bees any nectar (carbohydrates) or pollen (protein). [Sugar beet is a biennial plant. During the growing season in commercial sugar beet production, the crop produces a large storage root which contains significant amounts of sucrose as a carbohydrate storage source. If the plant would not be harvested at the end of the growing season, then during a potential 2nd growing season, nutrients and stored carbohydrate in the root will be used to produce flowers & seeds and the root will decrease in size. Thus, in commercial beet production, the root is harvested after the first growing season].

The same holds true for commercial cauliflower production: the commodity to be harvested as a vegetable is the white or greenish "head" of a cabbage crop, which is in fact the immature inflorescence of the plant. Thus, cauliflower is harvested before flowering (i.e. generally at about BBCH 49: "typical size, form and firmness of heads reached") and as such, bees are not attracted to the crop due to the lack of forage, i.e. up to and including BBCH 49, the vegetative crop stages do offer bees neither nectar (carbohydrates) nor pollen (protein).

Wheat, as a cereal crop, is in itself - at any growth stages - not a profitable foraging area for bees (no relevant nectar and/or pollen supplier). However, there are observations that honey bee forage in cereal crops in situations where aphid infestation attains a level that large amounts of honeydew are formed. However, high aphid infestation levels and associated formation of honeydew is not in compliance with Good Agricultural Practice, since it poses the risk of secondary infections, e.g. with sooty moulds and impacts yield quality. Accordingly, advisory use recommendations aim for product applications at infestation levels at which honeydew formation does not attain levels which attract honey bees.

There are 2 key application stages for aphid control:

- Fall treatments: Aphid control in fall is imperative to prevent virus infections. For efficient virus control, ARVALIS, the National Technical Institute for cereals in France calls for aphid control as soon as 10% of the plants bear aphids, or when aphids are encountered in the field for 10 consecutive days (http://www.arvalis-infos.fr/view.jspz;jsessionid=0982C13F8D2F8D3298E19B61C4107523E.tomcat1?obj=arvarticle&id=14339&syndtype=null&hasCookie=false&hasRedirected=true).
  10% of infested crop plants describe a very early stage of infestation, at which virus transmission can still be efficiently controlled
- Spring treatments: Aphid control in spring is highly important to ensure quality of yield. For the spring treatment, ARVALIS calls for a aphid control treatment, if 1 out of 2 ears are infested by at least 1 aphid
  http://www.arvalis-infos.fr/view.jspz?obj=arvarticle&id=4610&syndtype=null&hasCookie=false&hasRedirected=true). This infestation threshold is acknowledged as a simple and relevant indicator for the start of aphid population growth, i.e. when honeydew formation is still negligible.

Therefore, it can be concluded for sugar beet, cauliflower and cereals that the risk posed to honey bees by a foliar spray treatment of these crops is low due to poor crop attractiveness (no relevant nectar and/or pollen supplier), and application practices following the principles of Good Agricultural Practice, i.e. product use according to recommended pest threshold concepts, which keep aphid infestation below bee-attractive honeydew levels.

Nevertheless, in exceptional cases, honey bees may be attracted to those crops, e.g. during very dry and warm spring seasons with rapidly evolving pest-aphid populations, or due to high infestation levels of other pest species producing honeydew. Therefore, the following chapters address the risk

posed by deltamethrin foliar applications in those worst case scenarios. However, it must be stressed that honeydew-related bee incidents are reported for crops with higher pest threshold levels, and as such with a higher risk of honeydew formation - like potatoes, however, these cases are very incidental in crops like sugar beet, cauliflower and cereals.

Hazard Characterization of Deltamethrin

Deltamethrin acts on insect nerve membranes by delaying/inhibiting the closing of the activation gate for sodium ion channels. As a so called Type II pyrethroid, the inhibition of the sodium channel activation gate results in prolonged permeability of the nerve to sodium, which results in a series of repetitive nerve signals, i.e. in an overstimulation. This overstimulation swiftly paralyzes the insect's nervous system which in turn results in a fast knock-down effect, primarily induced via contact activity. In insects, de-toxification occurs metabolically via de-esterification, catalyzed by both, esterases and cytochrome P450 enzymes and via hydroxylation of aromatic rings, again by cytochrome P450 enzymes.

In standard laboratory toxicity assays, deltamethrin reveals a high intrinsic acute toxicity to honey bees. However, the extremely low contact toxicity endpoint as reported by [redacted] (1991; M-149380-01-1) for technical grade deltamethrin appears not to be reproducible. Such a low value was never recorded in either earlier studies (Anonymous , 1977; M-059919-01-1 and [redacted] 1978; M-098831-01-1) or in any study conducted afterwards. The study of [redacted] (2013; M-444971-01-1) is regarded as providing the most relevant endpoint, since the study was conducted with the actual specification of the technical grade deltamethrin and testing complied with the latest state-of-the art OECD-testing guidelines. The results of this latest study with technical grade deltamethrin (M-444971-01-1) did not show a higher toxicity of the technical material as compared to the EW 15 formulation in particular as well as to other investigated straight formulations. Moreover, when considering the inherent variability of the acute honey bee toxicity test system within a laboratory as well as inter-laboratory variability, this endpoint is well in line with all other reported endpoints except that of [redacted] (1991). A comparison of contact and oral toxicity endpoints suggests that deltamethrin is more toxic to bees via the contact than via the oral route (see Table 10.3.1- 1 and 10.3.1- 3), which is well line with the mode of action of deltamethrin (see chapters on product efficacy).

Due to the generally higher sensitivity of honey bees via the contact route of exposure and because the oral test system of bumble bees is currently lacking standardisation, bumble bees were exposed in the laboratory via the contact route in order to benchmark their susceptibility to deltamethrin against honey bees (Table 10.3.1- 2). The comparison of endpoint data as obtained with technical grade deltamethrin between bumble bees ([redacted] 2014; M-477381-01-1) and honey bees (Table 10.3.1-1) suggests that bumble bee foragers are less susceptible to deltamethrin than honey bee foragers.

In addition to the acute toxicity studies - where honey bees were one-time orally exposed to deltamethrin dissolved in small quantities of sugar solution, toxicity of a repeated oral exposure was also investigated. In this chronic toxicity study, honey bees were fed *ad libitum* at different treatment levels, exclusively with deltamethrin-spiked sugar syrup over 10 consecutive days ([redacted] (2014), M-477250-01-1). Due to the very low water solubility of technical grade deltamethrin, the study was conducted with the representative formulation, i.e. Deltamethrin EW 15. Chemical analysis confirmed a good agreement between actual and nominal treatment levels. The repeated oral exposure resulted in a 50% lethal dietary dose (LDD_{50}) of 0.53 µg a.s./bee/d. When comparing this 10-day LDD_{50} with the standard oral LD (lethal-one-time-dose)$_{50}$, of Deltamethrin EW 15 (Table 10.3.1- 3: 0.07 / 0.29 µg

a.s./bee), it can be concluded that deltamethrin has no delayed, chronic or accumulative toxic effects to honey bees.
In summary, deltamethrin shows a high intrinsic toxicity to honey bees. Results from standard laboratory toxicity assays clearly suggest that (formulated) deltamethrin poses a higher risk to honey bees by contact than by the oral route of exposure. Repeated oral exposure tests revealed that deltamethrin has no delayed, chronic or accumulative toxic effects to honey bees. Accordingly, risks posed by deltamethrin to honey bees are mainly related to short-term peak exposures which prevail during and shortly after foliar application. Under standardized laboratory conditions, bumble bee foragers were found to be less susceptible to deltamethrin than honey bee foragers. Therefore, and considering the intrinsic properties of deltamethrin (see below), product uses which pose a low risk to honey bees can be expected not to pose a relevant risk to bumble bees either.

Exposure Characterization of Deltamethrin

The very low vapour pressure of deltamethrin (≈1.2 ×10^{-8} Pa) renders deltamethrin to be practically non-volatile. Due to deltamethrin's high octanol-water partitioning coefficient ($\log P_{OW}$ ≈ 6) and low water solubility (≈ 0.2 µg/L), deltamethrin is virtually non-systemic. Accordingly, there will be no prolonged exposure of bees to residues in nectar and pollen after foliar application. This conclusion is strongly supported by field residue surveys which analysed nectar (honey) and pollen stores under realistic agronomic conditions, e.g. [redacted], 2006, 2011; [redacted] 2011; [redacted]. 2013.

From the hazard studies above and the physical-chemical properties of deltamethrin, it can be concluded that any intoxication risks for bees are mainly related to contact with spray residues, either by direct overspray or by rather fresh deposits. These risks exist during application and only short-term post-treatment. Due to deltamethrin's low solubility in aqueous media, its high lipophilicity which results in a very strong adsorptivity to organic surfaces, the lack of systemicity and the lack of volatility, it can be concluded that bee-pollinators will not encounter a chronic exposure situation from the label-compliant agronomic use of deltamethrin foliar products. This conclusion is strongly supported by the findings of [redacted] 1992 (M-166394-01-1) who determined the residual toxicity of deltamethrin on alfalfa foliage. When applied with a rate of 22.4 g a.s./ha, the RT_{25} , i.e. the time needed to cause mortality rates less than 25%, was less than 8 hours.

Risk Characterization from Forced Exposure Studies (Tunnel Studies)

A high number of tunnel studies have been conducted with various formulations, containing deltamethrin as the sole active substance, including Deltamethrin EW 15, which followed the respective current versions of either the French CEB-or the EPPO 170 guideline (see Table 10.3.1- 6). As indicated above, there is no indication that existing straight formulations of deltamethrin differ significantly in their intrinsic toxicity to honey bees. Under forced exposure conditions, an application range between 5 and 12.5 g a.s./ha formulated deltamethrin to the highly bee-attractive surrogate crop *Phacelia*, during honey bees actively foraging on the full-flowering crop, did in most cases result in only a slightly to moderately increased mortality, usually observed within the first 24 hours after application. Application rates between 7.5 and 12.5 g a.s./ha deltamethrin also caused a repellence effect, which was lasting for at least 24 hours after application. This repellence effect is well known for pyrethroid insecticides and is considered as a protective property of this chemical class for honey bees, since this effect will reduce honey bee exposure in/to treated areas. Further, the repellence effect is apparently not long-lasting and, therefore, is not expected to have a major impact on the food flow to the beehive.

The slightly to moderately increased initial mortality was also very short-lived and did not result in any observable adverse effect at colony level endpoints such as total brood-, food- and population development, or overall colony vitality. The same conclusion has been drawn from the most recent OECD GD 75 tunnel study which paid particular attention to bee brood development.

These findings are also in line with the results obtained in tunnel studies where deltamethrin was applied at an application rate of 6.25 g a.s./ha to sugar-treated cereals, simulating a worst-case honey dew exposure situation (see Table 10.3.1- 7). These studies followed the provisions of the respective current version of French CEB-guidelines under consideration of the provisions of the respective current version of the EPPO 170 guideline, and revealed only in some cases a slight increase in mortality within 24 hours after application, sometimes accompanied by a slight depression of foraging activity. The findings of these guideline-compliant studies are fully in-line with earlier non-GLP tunnel studies (see Table 10.3.1- 9), which did also not find any significantly increased mortality or adverse effects on brood development for application rates of 6.25 g a.s./ha and 12.5 g a.s./ha.

Two tunnel studies were conducted with bumble bees. Deltamethrin was applied at an application rate corresponding to12.5 g a.s./ha to full flowering *Phacelia* during bumble bees actively foraging on the crop (see Table 10.3.1- 8). The findings of the two tunnel studies are consistent and in-line to the findings of the laboratory study (Table 10.3.1- 2), indicating that foraging bumble bees are less susceptible to deltamethrin than honey bees.

The findings from forced exposure studies consistently support the risk predictions from the exposure and hazard characterization of deltamethrin. Even under forced exposure conditions, a foliar treatment of crops which are highly attractive to honey bees has only a very short-term effect on mortality and foraging activity, which was consistently found to have no adverse effect at the bee colony level. When applied during periods when honey bees were not actively foraging on the crop (e.g. during late evenings / early mornings), no adverse effects even at the level of individual bees could be observed. This finding is in line with the predominantly contact route of action and the rather short RT_{25}-values measured on treated foliage ([redacted] 1992; M-166394-01-1).

Risk Characterization from Realistic Exposure Studies (Field Studies)

In addition to the high number of studies conducted under forced exposure conditions, a series of field studies have also been conducted to characterize the risk to honey bees and honey bee colonies under realistic exposure conditions. In early non-GLP trials, conducted in the late 1970's and early 1980's, deltamethrin was applied at application rates between ≤ 7.5 up to and including 35 g a.s./ha to various bee-attractive crops during flowering and during bees actively foraging in these crops (see Table 10.3.1- 9). These trials revealed that deltamethrin, applied at application rates of ≤ 12.5 g a.s./ha, does not cause increased mortality even when applied to bee-attractive and full-flowering crops, while honey bees were actively foraging. An application rate of 17.5 g a.s./ha during bee flight caused only a very slightly increased mortality, application rates of 21.2 and 25 g a.s./ha during bee flight were found to be about the threshold rates for mortality. Only very high rates such as 35 g a.s./ha, applied to bee-attractive crops during honey bees actively foraging during full-bloom, resulted in a substantially increased mortality.
Up to and including 17.5 a.s./ha, there was a transient depression of foraging activity recorded (repellent effect), which was less prominent at application rates >17.5 g a.s./ha.
In some studies brood development, honey production and overwintering performance of exposed hives were also examined and no adverse effects were recorded for these colony level endpoints.

Where honey and/or pollen was analytically investigated, deltamethrin residues were either not detected or at only minute levels.
Besides these early non-GLP studies, further field studies are available which followed the respective current versions of EPPO 170 guideline , i.e. EPPO 170 (2) [1992], EPPO 170 (3) [2001] and EPPO 170 (4) [2010]. In these field studies, deltamethrin was applied at application rates of 7.5 and of 12.5 g a.s./ha on bee-attractive and full-flowering crops, while honey bees were actively foraging. One study was conducted at an application rate of 17.5 g a.s./ha, applied after daily bee flight. These guideline-compliant field studies confirm that deltamethrin has, if any, only slight to moderate effects on individual honey bees which are actively foraging in treated crops during application (see Table 10.3.1- 6). Mortality was in most cases not and in a few cases only marginally increased and no adverse sub-lethal or adverse behavioural effects could be observed. A slight, short-term depression of foraging intensity in the treated crop could be observed in some cases, generally limited to the period right after application. Most importantly, no adverse effects on hive vitality and brood/colony development were recorded.

In one field study ([redacted] (2007), M-295800-01-1), comprising three independent trials at different locations, also detailed investigations on the homing behaviour were made following an application of 7.5 g a.s./ha to full-flowering oil-seed rape (OSR) while honey bees were actively foraging. The data consistently revealed that there was no indication of any disturbance of the homing behaviour.

In two further identically designed field studies, Deltamethrin EW 15 was repeatedly applied to the highly bee-attractive surrogate crop *Phacelia tanacetifolia* ([redacted] (2012), M-452717-01-1 and M-452723-01-1) at 12.5 g a.s./ha with an application interval of 13 days. At both treatment days, honey bees were actively foraging within the full-flowering crop during application. Each of the two studies comprised one treatment field (T) and one control field (C), with 6 honey bee colonies per field, respectively. The field size was within the range of 2.1 - 2.3 ha and treatment-fields were separated from control fields by at least 4 km. The 1st application was conducted at BBCH 64-65 (during bees actively foraging) and the 2nd application at BBCH 65(-67), always during bees actively foraging. The colonies remained at the treated fields until end of flowering (BBCH 69), which resulted in a exposure duration of the colonies to the treated crop from 21 June - 21 July (1st study) and from 15 June - 15 July (2nd study). Thereafter, the colonies were evaluated in regular intervals for the rest of the season until overwintering, and a last time after overwintering. In both studies, assessments of acute (e.g. mortality, flight intensity, etc.) and chronic effects (e.g. season-long assessment of colony performance, food storage behaviour, health status, etc.) in comparison to the corresponding untreated control were conducted. Additionally, there were intensive, season-long assessments of colony health parameters, i.e. occurrence of bee diseases, viruses, *Nosema/Varroa*, etc. and finally an assessment of potential interactions with the overwintering performance. Both of these two long-term honey bee health studies consistently showed no treatment-related adverse effects on mortality, flight intensity or bee behavior throughout the entire field exposure period. Moreover, also no treatment-related adverse effects were observed on honey bee health, colony development (including colony strength, colony health, brood and food development) or overall colony vitality throughout the entire field exposure period and throughout the entire monitoring period including overwintering success.

All findings from field studies conducted with various commercial deltamethrin straight formulations consistently confirm the conclusion drawn from studies conducted under forced exposure conditions: Deltamethrin can be applied at rates of 12.5 g a.s./ha or lower during the daily bee flight activity to

bee-attractive crops without posing any undue risk to honey bees and honey bee colonies. All studies, either conducted under forced or realistic exposure conditions, have consistently demonstrated that adverse effects at colony level do not occur. Even repeated and prolonged exposure has neither resulted in adverse effects on colony health (occurrence of bee diseases, viruses, *Nosema/Varroa*, etc.) nor on adverse effects on overwintering performance of the exposed honey bee colonies.

Further Information from Published Research

1. Carvalho, S. M. [Reprint Author]; Carvalho, G. A.; Carvalho, C. F.; Bueno Filho, J. S. S.; Baptista, A. P. M. Toxicity of acaricides/insecticides for citrus crop to the africanized honey bee *Apis mellifera* L., 1758 (Hymenoptera: Apidae) . ORIGINAL TITLE: Toxicidade de acaricidas/insecticidas empregados citricultura para a abelha africanizada *Apis mellifera* L, 1758 (Hymenoptera: Apidae) (Doc. No.: M-461215-01-2)
2. Ramirez-Romero, R.; Chaufaux, J.; Pham-Delegue, M-H. (2005). Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. Apidologie, 36, 4, p. 601-611 (Doc. No.: M-460897-01-1)
3. Song, H.; Zhou, T.; Wang, Q.; Dai, P.; Luo, Q.; Xu, S.; Wu, Y. (2011). Effects of sublethal doses of insecticides on olfactory sensitivity of honeybee (*Apis mellifera ligustica*). Yingyong Kunchong Xuebao, 48, 3, p. 611-615 (Doc. No.: M-462163-01-2)
4. Dai, Ping-Li; Wang, Qiang; Sun, Ji-Hu; Liu, Feng; Wang, Xing; Wu, Yan-Yan; Zhou, Ting. (2010). Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee *Apis mellifera ligustica*. Environ. Toxicol. Chem., 29, 3, p. 644-649 (Doc. No.: M-461225-01-1)
5. A.E. Gradish, C.D. Scott-Dupree, A.J. Frewin, G.C. Cutler (2012). Lethal and sublethal effects of some insecticides recommended for wild blueberry on the pollinator *Bombus impatiens*. Can. Entomol. 144: 478–486 (Doc. No.: M-462175-01-1)
6. Carvalho, S. M.; Belzunces, L. P.; Carvalho, G. A.; Brunet, J.-L.; Badiou-Beneteau, A. (2013). Enzymatic biomarkers as tools to assess environmental quality: a case study of exposure of the honeybee *Apis mellifera* to insecticides. Environ. Toxicol. Chem, 32, 9, p. 2117-2124 (Doc. No.: M-464768-01-1)

Publication 1-4 (Carvalho, S. M et al, M-461215-01-2; 2. Ramirez-Romero, R.;et al. 2005, M-460897-01-1; Song, H. et al, 2011, M-462163-01-2; Dai, Ping-Li et al, 2010; M-461225-01-1) investigated honey bees with either individuals dosed under laboratory / indoor conditions (Carvalho, S. M et al, M-461215-01-2;. Ramirez-Romero, R.;et al. 2005, M-460897-01-1; Song, H. et al, 2011, M-462163-01-2; and queen dosing in Dai, Ping-Li et al, 2010; M-461225-01-1) or colonies, fed with defined doses under field conditions (Dai, Ping-Li et al, 2010; M-461225-01-1). Individual honey bees which were dosed under laboratory / indoor conditions suffered from various adverse effects in a dose-dependent manner. However, the ability of large honey bee colonies to absorb impacts from stressors and continue to grow makes extrapolation from effects observed in a laboratory on individual bees to effects of relevance at the colony level in the field extremely difficult. In many cases, acute risks of lethal or sub-lethal effects for individual bees observed in the laboratory may have no consequences whatever for colonies in the field. In summary, testing on individual bees does not allow for an

evaluation of potential impacts on the entire colony: only on the basis of field and monitoring studies is it possible to determine whether a particular stressor gives rise to a colony level impact.

Only Dai, Ping-Li et al (2010; M-461225-01-1) examined effects on colony level under field conditions. However, the authors applied deltamethrin at a total amount of 8,640 µg to each test colony per day which represents approximately 100 LC_{50} doses per colony per day over 20 days. On top, they dosed the queen each 5th day with a defined dose. No efforts were made to relate these doses to field-relevant doses. From the physico-chemical properties of deltamethrin it is very unlikely that colonies are exposed to deltamethrin over longer periods and the high lipophilicity makes is very unlikely that nurse bees feed queens with doses applied in this study. Finally, all (semi-)field studies which had been conducted with field-relevant application rates under realistic exposure conditions, i.e. spray treatment of flowering target crops, demonstrated that colonies were not impacted in any endpoint relevant for colony development and performance.

Gradish *et al.* (2012; M-462175-01-1) examined the effect of deltamethrin on bumble bees (*Bombus impatiens*). From laboratory assays, they calculated an oral LC_{50} of 33.8 ng deltamethrin/L (= 845 µg a.s./bee) which is substantially above the reported contact LC_{50} of 36 µg a.s./bee for *Bombus terrestris*, and suggests that deltamethrin acts primarily via the contact route as described for honey bees. The authors reported significantly reduced survival and reproduction rates of worker bees in micro-colonies which had been fed under laboratory conditions with 17 mg a.s./L deltamethrin (≈ 40 µg a.s./bee). No significant reduced survival or reproduction rates were observed for worker bees in micro-colonies which had been fed under laboratory conditions with 1.7 mg a.s./L deltamethrin (≈ 4 µg a.s. /bee). No attempt had been made to relate the applied doses to field-relevant doses. Two tunnel studies with field-relevant application rates (12.5 g a.s./ha) under realistic exposure conditions, i.e. spray treatment of flowering target crops, demonstrated that foraging bumble bees were not impacted in any endpoint investigated.

Carvalho *et al.* (2013; M-464768-01-1) exposed honey bees under laboratory conditions to lethal and sub-lethal doses of deltamethrin via the contact route of exposure. The 48h-LD_{50} of technical grade deltamethrin was found to be 0.051 µg a.s./bee, which is in line with the regulatory database. Sub-lethal dose of deltamethrin (LD_{50}/20 and LD_{50}/10) were found to have a short-term knock-down effect with a full recovery of the bees after 1 - 2 hours after exposure. The investigated sub-lethal doses of deltamethrin resulted in a modulation of some of the investigated biomarkers without inducing mortality.

In addition, several publications were found considering residues of deltamethrin in bee-relevant matrices. The following publications are considered as supplemental information regarding the exposure profile of deltamethrin to bee-pollinators:

1. Cossu, M.; Alamanni, M. C. (2003). Monitoring of pyrethroid residues in Sardinian honey by solid phase extraction and high-performance liquid chromatography. Ital. J. Food Sci., 15, 4, Page 541-551 (Doc. No.: M-457696-01-1)
2. Campillo, N.; Penalver, R.; Aguinaga, N.; Hernandez-Cordoba, M. (2006). Solid-phase microextraction and gas chromatography with atomic emission detection for multiresidue determination of pesticides in honey. Anal. Chim. Acta, 562, 1, p. 9-15 (Doc. No.: M-460886-01-1)

3. Chauzat, M.-P.; Faucon, J.-P.; Martel, A.-C.; Lachaize, J.; Cougoule, N.; Aubert, M. (2006). A survey of pesticide residues in pollen loads collected by honey bees in France. J. Econ. Entomol., 99, 2, Page 253-262 (Doc. No.: M-455906-01-1)

4. Chauzat, M.-P. ; Martel, A.-C.; Cougoule, N.; Porta, P.; Lachaize, J.; Zeggane, S.; Aubert, M.; Faucon, J.-P. (2011). An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. Environ. Toxicol. Chem, 30, 1, p. 103-111 (Doc. No.: M-455993-01-1)

5. Wiest, L.; Bulete, A.; Giroud, B.; Fratta, C.; Amic, S.; Lambert, O.; Pouliquen, H.; Arnaudguilhem, C. (2011). Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. J. Chrom., A 1218, 34, p. 5743-5756 (Doc. No.: M-456064-01-1)

6. Lambert, O.; Piroux, M.; Puyo, S.; Thorin, C.; L'Hostis, M.; Wiest, L.; Bulete, A.; Delbac, F.; Pouliquen, H. (2013). Widespread Occurrence of Chemical Residues in Beehive Matrices from Apiaries Located in Different Landscapes of Western France. PLoS ONE (17 Jun 2013) 8(6): e67007; DOI-No.: 10.1371/journal.pone.0067007 (Doc. No.: M-465046-01-1)

Cossu & Alamanni, M. C. (2003) in Italy as well as Campillo *et al*. (2006) in Spain investigated together 80 honey samples obtained from beekeepers, local markets and supermarkets for the presence of deltamethrin and found no quantifiable deltamethrin residues.

Chauzat *et al*. (2006) investigated the residue situation of several plant protection products in pollen loads collected by honey bees in France. Apiaries were evenly distributed in five sites located on continental France. Five colonies were randomly selected in each apiary, leading to a total of 125 studied honey bee colonies. For 3 year (starting in autumn 2002), colonies were visited four times per year: after winter, before summer, during summer and before winter. Pollen loads from traps were collected at each visit. In total, 82 pollen samples were subjected to deltamethrin residue analysis and in all samples no quantifiable deltamethrin residues were found.
In 2011, Chauzat *et al*. investigated several hive matrices like honey, pollen and bee-wax. The studied apiaries were distributed among five sites in continental France covering the main zones of French honey production. Professional and hobbyist apiarists took part in the investigation. At the beginning of the study, 125 colonies (five honey bee colonies randomly selected in five apiaries from five different locations across France) were pesticide residues over 3 year (2002-2005). Out of 198 analysed pollen samples, quantifiable deltamethrin residues were found only in one single sample (0.5% of all samples investigated). Out of 237 analysed honey samples, collected from the hives under investigation, quantifiable deltamethrin residues were found only in two samples (0.8% of all samples investigated). Out of 87 analysed bee-wax samples, collected from the hives under investigation, quantifiable deltamethrin residues were found only in one single sample (1.1% of all samples investigated).

Wiest *et al*. (2011) investigated bee-matrix samples collected in France during the beekeeping season 2008 and 2009. The samples were collected from16 apiaries of the "Région des pays de la Loire" (Western France) located in four types of landscapes (bocage, large-scale farming, gardening/ orchards, urban area) and two control apiaries (less inhabited landscapes) located in Atlantic islands (Island of Yeu and Island of Ouessant) . For each period, samples were collected in several colonies of every apiary (honey, foraging bees and trap pollen). No quantifiable deltamethrin residues were found.

Lambert *et al*. (2013) most likely refers to the same dataset as Wiest *et al*. (2011), and reassured that no quantifiable deltamethrin residues were found in all investigated matrices.

Overall, the findings of these publications, investigating the residue situation of deltamethrin in bee-relevant matrices, are well in line to findings made in regulatory investigations, and confirm that the physical-chemical properties of deltamethrin (virtually non-systemic, highly lipophilic and non-volatile) results in a favorable exposure profile.

Summary Conclusion

For deltamethrin, a very comprehensive database exists. Across all time scales, i.e. acute, short-term and long-term, deltamethrin shows a consistent hazard, exposure and risk profile:

- Deltamethrin acts on bees on the acute time scale, mostly via the direct contact route of exposure, and consistently across all short-and long-term studies, no delayed or chronic effects were recorded, neither on individual honey bees nor on the colony level,
- Foraging bumble bees were found to be less sensitive than foraging honey bees,
- The physical-chemical properties of deltamethrin (virtually non-systemic, highly lipophilic and non-volatile) results in a favourable exposure profile, i.e. a short period of bioavailability, reflected in very rare detects of deltamethrin residues in pollen and/or nectar(honey) in definitive studies and monitoring exercises,
- A large number of semi-field (forced exposure) and field (realistic exposure) studies, including studies investigating repeated and prolonged exposure scenarios, have consistently demonstrated that honey bees at the colony level are not at undue risk from foliar treatments of deltamethrin at field-relevant application rates. There is no indication that long-term honey bee health and/or the overwintering performance of colonies is impaired by a realistic worst-case exposure to deltamethrin,
- Sugar beet, cauliflower and cereal crops are not attractive to bees since these crops are no (relevant) nectar and/or pollen supplier. Attraction of bees to these crops by honeydew is a rare scenario, since use recommendations aiming to prevent virus infections (fall season) and to obtain high quality yields (spring season) direct farmers to apply the product well before aphid colonies have reached an infestation level where massive honeydew formation occurs. Accordingly, the risk, respectively the corresponding exposure situation, posed by deltamethrin foliar uses in sugar beet, cauliflower and cereal crops is substantially lower than in the highly bee-attractive surrogate crops used in the higher tier studies and provide additional margins of safety,
- Finally, deltamethrin is used since more than three decades in European agriculture in a range of bee attractive crops. Based on the available data from bee incident schemes and the long-lasting use experience, the compound can be regarded as fully compatible with apicultural operations.

Overall, it can be concluded that the use of deltamethrin in sugar beet, cauliflower and cereals 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

Report:	**KCP 10.3.1.1.1/01, [redacted] (2003)**
Title:	Acute toxicity of Deltamethrin Protec EW 15 to the honeybee *Apis mellifera* L. under laboratory conditions
Document No:	M-103549-01-1 (Rep. No.: 031048047)
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes

Material and Methods:

The insecticide Deltamethrin Protec EW 15 (AE F032640 00 EW01 B113, analytical content: 16.05 g/L (equivalent to 1.64 % w/w) deltamethrin (AE F032640); specification: Article No.: 0308474, Batch: AAIM00846), was tested under laboratory conditions on the honey bee *A. mellifera* after oral and contact exposure. Endpoints were mortality and behaviour of the bees compared to control up to 48 h after application. Mortality values were used to provide a regression line and to calculate the median lethal dose value (LD_{50}) expressed in µg of active ingredient or product per bee.

Application rates for contact and oral toxicity test (values in brackets based on the actual consumed amount of sucrose solution)

Contact toxicity		Oral toxicity	
µg product/bee	µg a.s./bee	µg product/bee	µg a.s./bee
38.2	0.6	38.2 (36.18)	0.6 (0.568)
19.1	0.3	19.1 (16.85)	0.3 (0.264)
9.55	0.15	9.55 (9.17)	0.15 (0.144)
4.78	0.075	4.78 (4.76)	0.075 (0.075)
2.39	0.038	2.39 (2.38)	0.038 (0.037)

Toxic standard Dimethoate EC 400 was applied at the following doses

Contact toxicity		Oral toxicity	
µg product/bee	µg a.s./bee	µg product/bee	µg a.s./bee
0.663	0.250	0.663	0.250
0.332	0.125	0.331	0.125
0.166	0.062	0.166	0.062
0.083	0.031	0.083	0.031

Findings:

The study was performed in compliance with the GLP principles.

The validity criterion - mortality in the control ≤ 10 % - was accomplished (being 0 % in the contact and oral toxicity tests after 48 hours). The LD_{50} (24 h) values for the toxic standard of 0.1 - 0.30 µg a.s./bee (contact) and 0.1 - 0.35 µg a.s./bee (oral) were accomplished (being 0.233 µg a.s./bee and 0.139 µg a.s./bee in the contact and the oral toxicity tests, respectively).

Oral and contact toxicity LD_{50} values of bees treated with Deltamethrin Protec EW 15

Test item	Deltamethrin Protec EW 15				
Test object	Honeybee *Apis mellifera L.*				
Exposure	contact / oral				
Treatment	LD_{50}				
	time	contact toxicity test		oral toxicity test	
		µg product/bee	µg a.s./bee	µg product/bee	µg a.s./bee
Test item Deltamethrin Protec EW 15	24 h 95 %-cl lower upper	4.978 4.186 5.920	- -	7.308 3.390 5.475	- -
	48 h 95 %-cl lower upper	4.768 4.001 5.682	- -	4.069 3.170 5.222	- -
Reference item Dimethoate EC 400	24 h 95 %-cl lower upper	-	0.233 0.212 0.256	-	0.139 0.116 0.166
	48 h 95 %-cl lower upper	-	0.171 0.146 0.201	-	0.135 0.113 0.162

cl: confidence limits

Observations:

No statistically significant effects of the test item Deltamethrin Protec EW 15 on survival were observed at the dose of 2.39 µg product per bee in the contact toxicity test (10 % mortality) during 48 hours. For the tested doses of 4.78, 9.55, 19.1 and 38.2 µg product per bee statistically significant effects of the test item on survival were observed (56.7, 83.3 and 100 % mortality, respectively) during 48 hours. The calculated LD_{50} (48 h) was 4.98 µg product per bee in the contact toxicity test.
Statistically significant effects on survival were observed at consumed doses of 2.38, 4.76, 9.17, 16.85 and 36.18 µg product per bee in the oral toxicity test (26.7, 63.3, 73.3, 96.7 and 100 % mortality, respectively) during 48 hours. Therefore, the calculated LD_{50} (48 h) was 4.07 µg product per bee in the oral toxicity test.
Before bees died in the test item treatments, apathy and immobility were observed shortly after application until 24 hour assessment.

Conclusions:

Therefore, the calculated LD_{50} (48 h) was 4.77 µg product/bee (equivalent to 0.078 µg a.s./bee) in the contact toxicity test and 4.07 µg product/bee (equivalent to 0.067 µg a.s./bee) in the oral toxicity test.

*****

Report:	**KCP 10.3.1.1.1/02, [redacted] (2000)**
Title:	Oral toxicity (LD_{50}) to honey bees (*Apis mellifera* L.) Deltamethrin oil in water emulsion 15 g/L Code: AE F032640 00 EW01 B103
Document No:	M-199244-01-1 (Rep. No.: CW00/027)
Guidelines:	EPPO 170
GLP:	Yes

Material and Methods:
Groups of fifty worker honey bees, *Apis mellifera*, were offered 5 concentrations of the test material in a sucrose diet for 5 hrs; the tested concentrations were 0.039; 0.078; 0.155; 0.233 and 0.311 % (w/w) product. Actual food consumption was measured after 5 hrs, and then the numbers of dead bees in each cage were assessed after 24, 48, and 72 hrs.
Based on the quantities of food actually consumed during the 5 hr feeding period, the mean measured dose rates to which the bees were exposed were equivalent to 8.69; 12.01; 18.37; 22.26 and 34.26 µg product/bee.

Findings:

	AE F032640 00 EW01 B103
Time (hours)	LD_{50} (95 % fiducial limits) µg product per bee
24	22.737 (20.311 – 26.116)
48	20.392 (18.180 – 23.265)
72	19.857 (17.457 – 23.049)

*****

Report:	**KCP 10.3.1.1.1/03, [redacted] (2003)**
Title:	Acute toxicity of Deltamethrin EW 50 to the honeybee *Apis mellifera* L. under laboratory conditions
Document No:	M-270468-01-1 (Rep. No.: 05 10 48 103)
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	Yes

Material and methods
Test item: Deltamethrin EW 50 (AE F032640 50 EW), analysed content: 51.69 g/L deltamethrin; specification: Article No.: 00-0648136, Development No.: 30-00375503, Batch: 08398/0049(0031), TOX No.: 07112-00
Reference item (toxic standard): Perfekthion EC 400 (analysed content: Dimethoate: 408.7 g/L)
Deltamethrin EW 50 was tested under laboratory conditions on the honeybee *A. mellifera* after oral and contact exposure. Endpoints were mortality and behaviour of the bees compared to control up to 48 h after application.

Application rates for contact and oral toxicity test (based on analysed content of a.i.):

Contact toxicity		Oral toxicity*	
Test item [µg product/bee]	Deltamethrin [µg a.i./bee]	Test item [µg product/bee]	Deltamethrin [µg a.i./bee]
20.0	0.994	39.4	1.960
8.0	0.398	19.5	0.967
3.2	0.159	9.8	0.487
1.28	0.064	5.0	0.249
0.51	0.025	2.5	0.124

*based on actual intake

Applied/exposed volume in the contact test: 2 µL 0.1 % Tween solution/bee (according to the practical experience and to guarantee a good penetration of the test item this application volume is more appropriate than 1 µL/bee suggested in the guideline).
Applied/exposed volume in the oral toxicity test: 200 µL sucrose solution/10 bees = 20 µL/bee.

Toxic standard Perfekthion EC 400, applied at the following doses:

Contact toxicity		Oral toxicity*	
Reference item [µg product/bee]	Dimethoate [µg a.i./bee]	Reference item [µg product/bee]	Dimethoate [µg a.i./bee]
1.315	0.500	1.309	0.498
0.657	0.250	0.656	0.249
0.329	0.125	0.329	0.125
0.164	0.0625	0.164	0.0625

*based on actual intake

Applied/exposed volume in the contact test: 2 µL 0.1 % Tween solution/bee
Applied/exposed volume in the oral toxicity test: 200 µL sucrose solution/10 bees = 20 µL/bee

Findings

The validity criteria were met as mortality in the was control ≤ 10 % (being 0 % in the contact and oral toxicity tests after 48 hours) and the LD_{50} 24 h values for the toxic standard were in the postulated range of 0.1 - 0.30 µg a.i./bee (contact) and 0.1 - 0.35 µg a.i./bee (oral) (being 0.195 µg a.i./bee and 0.137 µg a.i./bee in the contact and the oral toxicity tests, respectively).

Oral and contact toxicity LD_{50} values of bees treated with Deltamethrin EW 50:

Test item	Deltamethrin EW 50				
Test object	Honeybee *Apis mellifera* L.				
Exposure	contact / oral LD_{50}				
	treatment time	contact toxicity test		oral toxicity test	
		µg product/bee	µg a.i./bee	µg product/bee	µg a.i./bee
Test item Deltamethrin EW 50	24 h 95 %-cl lower upper	2.6 2.1 3.3	- -	17.1 14.6 19.9	-
	48 h 95 %-cl lower upper	2.5 2.0 3.2	- -	15.9 13.0 19.5	-
Reference item Perfekthion EC 400	24 h 95 %-cl lower upper	- -	0.195 0.168 0.227	- -	0.137 0.121 0.150
	48 h 95 %-cl lower upper	- -	0.172 0.144 0.204	- -	0.132 0.116 0.149

cl: confidence limits

In the contact toxicity test no statistically significant effects of the test item Deltamethrin EW 50 on survival were observed at tested doses of 0.51 and 1.28 µg product per bee (3.3 and 16.7 % mortality, respectively) after 48 hours. For the tested doses of 3.2, 8.0 and 20.0 µg product per bee statistically significant effects of the test item on survival were observed (56.7, 96.7 and 100 % mortality, respectively) after 48 hours. The calculated LD_{50} (48 h) was 2.5 µg product per bee (equivalent to 0.12 µg a.i./bee) in the contact toxicity test.
In the oral toxicity test no statistically significant effects of the test item on survival were observed at consumed doses of 2.5, 5.0 and 9.8 µg product per bee (6.7, 6.7 and 10.0 % mortality, respectively) after 48 hours. For the consumed doses of 19.5 and 39.4 µg product per bee statistically significant effects of the test item on survival were observed (53.3 and 100 % mortality, respectively) after 48 hours. The calculated LD_{50} (48 h) was 15.9 µg consumed product per bee (equivalent to 0.79 µg consumed a.i./bee) in the oral toxicity test.

Observations
In the contact toxicity test no effects on behaviour were observed in honeybees after exposure to doses of 0.51 and 1.28 µg product per bee. Bees exposed to doses equal or greater than 3.2 µg product per bee were affected at the 4 hour assessments. At assessments conducted 24 and 48 hours after contact exposure bees had generally recovered and no different behaviour for all surviving bees exposed up to a dose of 8.0 µg product/bee compared to control bees was observed.

In the oral toxicity test no effects on behaviour were observed in honeybees consuming doses equal or less than 9.8 µg product/bee. After consuming doses of 19.5 and 39.4 µg product/bee most bees were affected at the 4 hour assessment. At the assessments conducted 24 and 48 hours after oral exposure affected bees had generally recovered and no different behaviour for all surviving bees consuming doses up to 39.4 µg product/bee compared to control bees was observed.

Conclusion

The calculated LD_{50} (48 h) was 2.5 µg product per bee (equivalent to 0.12 µg a.i./bee) in the contact toxicity test and 15.9 µg consumed product per bee (equivalent to 0.79 µg consumed a.i./bee) in the oral toxicity test

*****

Report:	**KCP 10.3.1.1.1/04, [redacted] (2000)**
Title:	Oral toxicity (LD_{50}) to honey bees (*Apis mellifera* L.) Deltamethrin emulsifiable concentrate 15 g/L
Document No:	M-199148-01-1 (Rep. No.: CW00/026)
Guidelines:	EPPO Guideline No.170
GLP:	yes

Material and Methods:

In this laboratory study the oral toxicity of the acaricide AE F032640 00 EC02 A804 to the worker honey bee was determined. The study was conducted in compliance with EPPO Guideline No.170 and the Principles of Good Laboratory Practice. Groups of fifty worker honey bees, *Apis mellifera*, were offered 5 concentrations of the test material in a sucrose diet for 5 hr; the concentrations tested were 0.35; 0.17; 0.09; 0.05 and 0.02 % (w/w) product. Actual food consumption was measured after 5hr, and then the number of dead bees in each cage were assessed after 24, 48 and 72 hours. Based on the quantity of food actually consumed during the 5 hr feeding period, the mean measured dose rates to which the bees were exposed were equivalent to 7.7, 13.4, 18.0, 23.7 and 29.7 µg product/bee.

Findings:

	AE F032640 00 EC02 A804
Time (hours)	**LD_{50} (95% fiducial limits) µg product per bee**
24	25.789 (21.173 – 43.043)
48	25.613 (20.624 – 47.700)
72	24.973 (20.210 – 41.000)

*****

Report:	**KCP 10.3.1.1.1/05, [redacted] (1987)**
Title:	Laboratory investigations into the toxicity of Hoe 032640 - emulsifiable concentrate 25 (g/l) (Code: Hoe 032640 0I EC03 A115) to the Honey Bee *Apis mellifera* L.
Document No:	M-118431-01-1 (Rep. No.: 1EA/I/87/005)
Guidelines:	none
GLP:	no

Material and Methods:

The effect of deltamethrin, emulsifiable concentrate 25g/L; Code of the test substance: AE F032640 00 EC03 B0, on worker honey bees, *Apis mellifera*, was investigated in a contact and oral toxicity test. In the contact test two trials, in the oral test three trials were conducted with 5 replicates containing 10 bees each. Dose rates in the contact test were 0.001, 0.005, 0.01 and 0.05 µg a.i./bee, in the oral test 0.01, 0.05, 0.1, 0.5 and 1.0 µg a.i./bee, respectively. The mortality was determined after 24 and 48 h.

Findings:

	Contact test	Oral test
Time (hours)	**LD_{50} µg a.s. per bee**	**LD_{50} µg a.s. per bee**
24	0.01	0.38
48	0.01	0.28

*****

Report:	**KCP 10.3.1.1.1/06, [redacted] (2008)**
Title:	Effects of Deltamethrin EC 25AF G (Acute Contact and Oral and Honey Bees) Apis mellifera L.) in the Laboratory
Document No:	M-309900-01-1 (Rep. No. 33041035)
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes

Material and Methods:

Deltamethrin EC 25AF G (Deltamethrin: 2.5 % w/w nominal, 2.77 % w/w analytical), Specification: Batch No.: 2008-000434; under laboratory conditions *Apis mellifera* 30 worker bees were exposed for 48 hours to doses of 1040, 450, 243, 136 and 67.5 ng a.i. per bee for feeding (oral dose response test, value based on the actual intake of the test item) and 30 worker bees per treatment were exposed for 96 hours to doses of 250, 125, 63, 31 and 16 ng a.i. per bee for topical application (contact dose response test). The contact toxicity test was prolonged for 48 hours due to increasing mortality between 24 and 72 hours, up to a maximum of 96 hours.

Test Item	Deltamethrin EC 25AF G	
Test object	*Apis mellifera*	
Application rate (ng a.s./bee)	1040, 450, 243, 136 and 67.5	250, 125, 63, 31 and 16
Exposure	oral (sugar solution)	contact (solution in Adhäsit (0.5 %)/water)
LD_{50} (ng a.s./bee)	24 hours: 151; 48 hours: 143	24 hours: > 250; 48 hours:138; 72 hours: 108; 96 hours: 110

Observations:

Contact Test:

Dose levels of 250, 125 and 63 ng a.s. per bee led to mortality ranging from 96.7 to 6.7 % at the end of the test (96 hours). No mortality occurred in the 31 and 16 ng a.s. dose groups. There was 3.3 % control (water + 0.5 % Adhäsit) mortality.

Behavioural abnormalities (e.g. moving coordination problems, apathy and/or vomiting) occurred in all treatment groups during the 4 hours assessment. 24 hours following the application these symptoms were still found in all treatment groups except the 16 ng a.s./bee dose level. During the 48 hours assessment some behavioural abnormalities still occurred but only in the two highest dose groups. After 72 and 96 hours in the highest dose group (250 ng/bee) a few apathetic bees were found only.

Oral Test:

Nominal dose levels of the 1000, 500, 250, 125 and 62.5 ng a.s./bee corresponded to an actual intake of 1040, 450, 243, 136 and 67.5 ng a.s./bee, respectively. Mortality occurred at 1040, 450, 243 and 136 ng a.s./bee in a dose related pattern. The oral dose levels resulted in mortality ranging from 100.0 to 46.7 % at the end of the test (48 hours after application). There was no mortality in the 67.5 ng/bee dose group. No mortality occurred in the control (50 % sugar solution). During the first 4 hours, behavioural abnormalities (*e.g.* movement coordination problems and/or apathy) were observed in all treatment groups with the exception of the 67.5 ng a.s./bee dose group. During the 24 hours assessment behavioural abnormalities of a few bees were still found among the dose groups. No further behavioural impairments were found at the 48 hours assessment. The contact and oral LD50 values of the reference item (dimethoate) were calculated to be 0.18 and 0.14 µg a.s./bee, respectively.

Conclusions:
The toxicity of Deltamethrin EC 25AF G was tested in both an acute contact and oral toxicity test on honey bees. The LD_{50} (24 h) value of Deltamethrin EC 25AF G was determined to be greater than 250 ng a.s./bee and the LD_{50} (48 h + 72 h + 96 h) values of Deltamethrin EC 25AF G were 138, 108, and 110 ng a.s./bee in the contact toxicity test, respectively. The LD_{50} (24 h + 48 h) values were 151 and 143 ng a.s./bee in the oral toxicity test, respectively.

*****

Report:	**KCP 10.3.1.1.1/07, [redacted] (2000)**
Title:	Oral toxicity (LD_{50}) to honey bees (*Apis mellifera* L.) Deltamethrin emulsifiable concentrate 100 g/L
Document No:	M-199150-01-1 (Rep. No.: CW00/028)
Guidelines:	EPPO Guideline No.170
GLP:	yes

Material and methods:
Groups of fifty worker honey bees, *Apis mellifera*, were offered 5 concentrations of the test material in a sucrose diet for 5 hrs; the tested concentrations were 0.33; 0.16; 0.08; 0.04; and 0.02% /w/w) product. Actual food consumption was measured after 5 hrs, and then the numbers of dead bees in each cage were assessed after 24, 48, and 72 hrs. Based on the quantities of food actually consumed during the 5 hr feeding period, the mean measured dose rates to which the bees were exposed were equivalent to 2.27; 3.63; 7.13; 13.0; and 20.20 µg product/bee.

Findings:

	AE F032640 00 EC11 A308
Time (hours)	LD_{50} (95 % fiducial limits) µg product per bee
24	2.935 (0.046 – 5.534)
48	2.563
72	2.534

Conclusion:
The oral LD_{50} was determined to be 2.534 µg product/bee after 72 h of exposure.

*****

Report:	**KCP 10.3.1.1.1/08, [redacted] (1995)**
Title:	Determination of the oral toxicity of Decis WG2 (Hoe 032640 00 EG06 A101) to the honey bee *Apis mellifera* L.
Document No:	M-134668-01-1 (Rep. No.: CW94/067E)
Guidelines:	BBA VI, 23-1, (Jun.1991)
GLP:	yes

Material and Methods:
The oral toxicity of AE F032640 00 EG06 A101 was investigated in the laboratory. The bees were fed for 5 hours with a 50% sugar solution containing the test substance at 0.002, 0.004, 0.008 and 0.016% a.i. (w/w). At the end of this time the amount consumed was checked by weighing. Afterwards the bees received a pure 50% sugar solution ad libitum instead of test substance until the end of the study. The checks were carried out 24, 48 and 72 hours after treatment. The LD_{50} was calculated by probit analysis.
Based on the quantities of food actually consumed during the 5 hr feeding period, the mean measured dose rates to which the bees were exposed were equivalent to 3.7; 5.2; 3.9; 4.7 µg a.i./bee.

Findings:

	AE F032640 00 EG06 A101
Time (hours)	**LD_{50} (95% fiducial limits) µg a.i. per bee**
24	0.192 (0.152 – 0.236)
48	0.182 (0.143 – 0.224)
72	0.182 (0.143 – 0.224)

*****

Supplemental information from the literature

Report:	**KCP 10.3.1.1.1/09 : Ramirez-Romero, R.; Chaufaux, J.; Pham-Delegue, M-H. (2005)**
Title:	Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee Apis mellifera, a comparative approach.
Source:	Apidologie, 36: 4, p. 601-611
DOI No:	10.1051/apido:2005039
Document No:	M-460897-01-1
Guidelines:	-
GLP:	

EXECUTIVE SUMMARY
The study compared the mortality, rate of syrup consumption, foraging activity and learning performances of free-flying honeybees from colonies fed with syrups containing Cry1Ab protoxin, deltamethrin or imidacloprid with bees from the same colonies given syrups without additives. In addition, to estimate the dynamic of Cry1Ab protoxin in the hive, we present data obtained when honey, larvae and bee foragers were analysed using immunological tests (ELISA). However, material and methods as well as results are summarized for deltamethrin only.
Experiments were performed on a colony of *Apis mellifera* L. (about 10 000 bees) with a one-year queen and 3 brood combs. Experiments were done in a flight cage (2.5 x 2 x 2 m) placed in an acclimatized room (23 ± 1°C, 50% RH, photoperiod: 12:12 (L:D), 400 lux artificial lighting during observation periods, and 200 lux after observation period). The sugar syrup (500 g/L sucrose)

contained 500 µg/kg deltamethrin (99% purity, sample: 81112; Cluzeau InfoLabo (Sainte-Foy La Grande, France)). No control experiment was performed. Three observation periods of 4 days each were used to investigate mortality, syrup consumption, foraging activity and olfactory learning performance. A disruption of 3 days, during which the colony was provided with non-contaminated sugar syrup, was allowed between each period to let the colony recover.
All dead bees found on the ground were counted over four consecutive days. Syrup was renewed daily. The standard feeding device (glass bottle) was positioned 1.5 m from the hive entrance. Syrup consumption was estimated daily by measuring the difference in volume on each of four consecutive days for each observation period.
The foraging activity and learning performances were evaluated using an artificial flower device described by Pham and Masson (1985)[5] and modified by Decourtye et al. (2004)[6]. During conditioning, the 6 artificial flowers were filled with syrup (contaminated or not) and were offered in association with a conditioning stimulus (pure linalool). When the flower device was used to test learning performance, the flowers did not contain the syrup and only three out of six flowers provided the odour stimulus (testing device). The flower device was placed 1.5 m from the hive entrance. To evaluate foraging activity, bees were allowed to visit the conditioning device over 4 days, for a 2 hours observation period the first 2 days, and 1 hour observation period the following 2 days. All bees spontaneously visiting the device during the observation periods were marked by a colour dot on the thorax and counted. After recording foraging activity, olfactory learning performance was tested. This testing procedure consisted of a conditioning phase (15 min) where the conditioning device was offered (and the new bees visiting the device were marked as in the period of foraging activity recording) alternating with a testing phase (5 min) using the testing device. The learning performance was evaluated by recording the number of marked bees visiting the flowers delivering the CS on the testing device. A total of 4 conditioning and 4 testing periods per day were conducted. Additionally, the volume of syrup administered during experiments was noted and the volume of remaining syrup was measured.
The mortality recorded before treatment was significantly higher than mortality recorded after treatment (χ^2=6.5; 2 df; P = 0.04) (Mean number per day: before treatment: 196.3, during treatment: 109.5 and after treatment 102.75). Syrup uptake was significantly lower during and after treatment than before treatment (F = 46.9; 2 df; P < 0.01) (Mean number per day: before treatment: 110.1 ml; during treatment: 44.8 ml and after treatment: 64.9 ml).
Foraging activity was significantly higher before treatment than during and after treatment (F = 11.7; 2 df; P < 0.01). The mean number of visits before treatment was 1.7 (± 0.3 SEM), 0.7 (± 0.1 SEM) during the treatment and 1.0 (± 0.1 SEM) after treatment. The decrease due to the treatment was of ca. 60% visits, and the recovery after treatment led to an increase of ca. 35% visits.
The olfactory learning performance was strongly reduced during the treatment period and the level of visits was not significantly different from a randomised distribution between scented and unscented sites (P = 0.1 and P = 0.5). Similarly, after treatment, the visits on scented sites were not significantly different from that on unscented sites (P = 0.1 and P = 0.1).

MATERIAL AND METHODS

[5] Pham M.H., Masson C. (1985) Analyse par conditionnement associatif du mécanisme de la reconnaissance de sources alimentaires par l'abeille, Bull. Soc. Entomol. Fr. 90, 1216–1223.
[6] Decourtye A., Devillers J., Cluzeau S., Charreton M., Pham-Delègue M.H. (2004) Effects of imidacloprid and deltamethrin on associative learning in honeybee under semi-field and laboratory conditions, Ecotoxicol. Environ. Saf. 57, 410–419.

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamehtrin
Adjuvant / Surfactant:	--
Source of test item:	[redacted]
Lot/Batch number:	*Sample:* 81112
Purity:	99%
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Acetone
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Apis mellifera* L.
Cultivar:	-
Source of test species:	-
Age of test organisms at study initiation / Crop growth stage at treatment:	Conlony with about 10 000 bees and a one year queen
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Mortality, Syrup consumption, foraging activity and olfactory learning performance
Duration of study:	Three observation perods of 4 days (before treatment, during treatment and after treatment) with a disruption of 3 days between each period.
Treatments:	Syrup containing deltamethrin and pure syrup
Test concentrations:	500 µg/kg
Number of replicates:	-
Individuals per replicate:	1 colony
Test units (type and size):	Flight cage (2.5 x 2 x 2 m)
Application - device / nozzles:	Syrup in a glass bottle or in an artificial flower device (1.5 m from hive entrance)
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Syrup (500 g/l sucrose)
Temperature / relative humidity:	23 ± 1°C, 50% RH,
Photoperiod:	12:12 (L:D)
Lighting	400 lux artifial lighting during observation periods, and 200 lux after observation periods
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-

Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality, Syrup consumption, foraging activity and olfactory learning performance
Measurement frequency:	Daily
Statistical analyses:	Friedman's analysis of variance test, non-parametric analysis of variance, repeated-measures analysis of variance and chi-square analysis

RESULTS

1. Validity criteria:

No validity criteria were stated.

2. Biological findings:

The mortality recorded before treatment was significantly higher than mortality recorded after treatment ($\chi 2$ =6.5; 2 df; P = 0.04) (Mean number per day: before treatment: 196.3, during treatment: 109.5 and after treatment 102.75). Syrup uptake was significantly lower during and after treatment than before treatment (F = 46.9; 2 df; P < 0.01) (Mean number per day: before treatment: 110.1 ml; during treatment: 44.8 ml and after treatment: 64.9 ml).

Foraging activity was significantly higher before treatment than during and after treatment (F = 11.7; 2 df; P < 0.01). The mean number of visits before treatment was 1.7 (± 0.3 SEM), 0.7 (± 0.1 SEM) during the treatment and 1.0 (± 0.1 SEM) after treatment. The decrease due to the treatment was of ca. 60% visits, and the recovery after treatment led to an increase of ca. 35% visits.

The olfactory learning performance was strongly reduced during the treatment period and the level of visits was not significantly different from a randomised distribution between scented and unscented sites (P = 0.1 and P = 0.5). Similarly, after treatment, the visits on scented sites were not significantly different from that on unscented sites (P = 0.1 and P = 0.1).

Table 1: Mortality in relation to treatment*. Data represent mean number of dead honeybees per day (± SEM) which were found on the ground of the flight chamber. Mortality was recorded over 4 days per week.

Before treatment	During treatment	After treatment
169.3a	109.5 ± 24.8ab	102.75 ± 16.3b

Different letters following the means within a row indicate significant differences.

Table 2: Syrup consumption for each treatment*. Data represent the mean value of syrup consumption (mL) per day (± SEM). Syrup consumption was recorded along 4 days per week.

Before treatment	During treatment	After treatment
110.1 ± 7.6a	44.8 ± 1.7 b	64.9 ± 5.6 b

Different letters following the means within a row indicate significant differences.

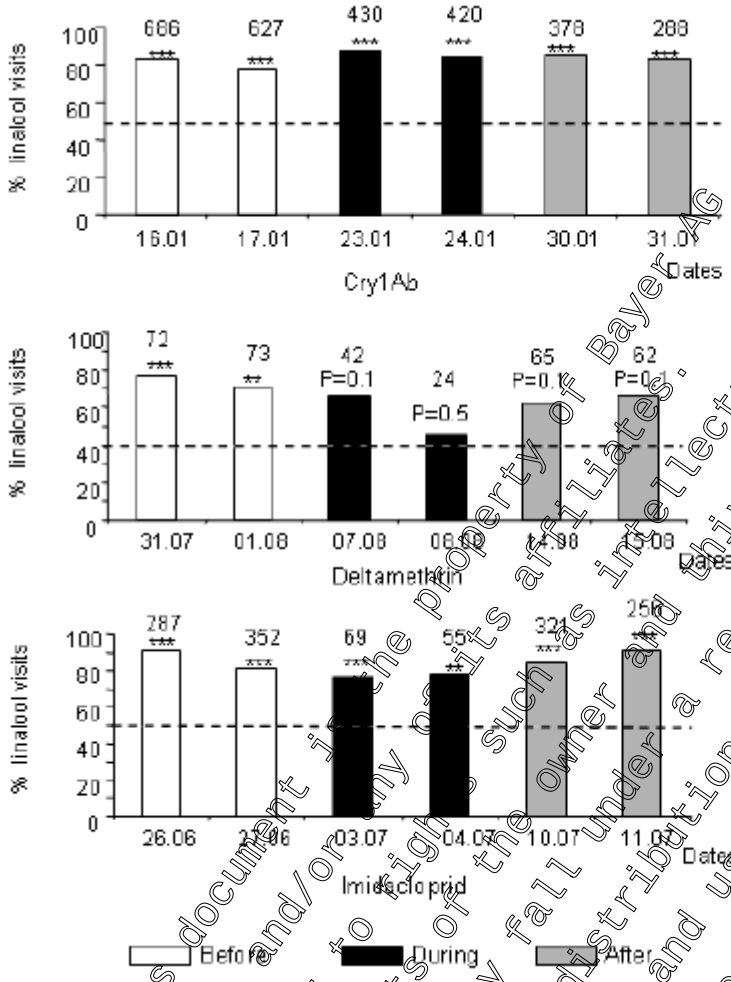


Figure 2. Olfactory learning performance of free-flying foragers in relation to treatment. After conditioning to linalool (scented sites + reward syrup), the visits of foragers on either the scented sites or the unscented ones were noted every 30-s during 3 periods of 5-min per day. Bars give the percentage of foragers visiting the scented sites after conditioning to linalool. White and grey bars correspond to the periods of feeding with the control syrup before and after the treatment, respectively. Black bars are related to the period with the contaminated syrup. Each testing period was conducted over 2 days, separated by 2 days of recording foraging activity and 3 days of inter-treatment recovery. The total number of foragers visiting the testing device is indicated above the bars. The observed numbers of visits were compared to a hypothetical equal distribution of landings on the scented sites and unscented sites, shown as the 50% dotted line. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

RESULTS SUMMARY

The mortality recorded before treatment was significantly higher than mortality recorded after treatment ($\chi 2 = 6.5$; 2 df; P = 0.04) (Mean number per day: before treatment: 196.3, during treatment: 109.5 and after treatment 102.75). Syrup uptake was significantly lower during and after treatment than before treatment ($F = 46.9$; 2 df; $P < 0.01$) (Mean number per day: before treatment: 110.1 ml; during treatment: 44.8 ml and after treatment: 64.9 ml).

Foraging activity was significantly higher before treatment than during and after treatment ($F = 11.7$; 2 df; $P < 0.01$). The mean number of visits before treatment was 1.7 (± 0.3 SEM), 0.7 (± 0.1 SEM) during the treatment and 1.0 (± 0.1 SEM) after treatment. The decrease due to the treatment was of ca. 60% visits, and the recovery after treatment led to an increase of ca. 35% visits.

The olfactory learning performance was strongly reduced during the treatment period and the level of visits was not significantly different from a randomised distribution between scented and unscented sites (P = 0.1 and P = 0.5). Similarly, after treatment, the visits on scented sites were not significantly different from that on unscented sites (P = 0.1 and P = 0.1).

Comment of the Notifier: Individual honey bees which were dosed under laboratory / indoor conditions suffered from various adverse effects in a dose-dependent manner. However, the ability of large honey bee colonies to absorb impacts from stressors and continue to grow makes extrapolation

from effects observed in a laboratory on individual bees to effects of relevance at the colony level in the field extremely difficult. In many cases, acute risks of lethal or sub-lethal effects for individual bees observed in the laboratory may have no consequences whatever for colonies in the field. In summary, testing on individual bees does not allow for an evaluation of potential impacts on the entire colony: only on the basis of field and monitoring studies is it possible to determine whether a particular stressor gives rise to a colony level impact.

Report:	**KCP 10.3.1.1.1/10; Dai, Ping-Li; Wang, Qiang; Sun, Ji-Hu; Liu, Feng; Wang, Xing; Wu, Yan-Yan; Zhou, Ping. (2010)**
Title:	Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera ligastica.
Source:	Environ. Toxicol. Chem., 29, 3, p. 644-649
DOI No: Document No:	10.1002/etc.67 M-461225-01-1
Guidelines:	-
GLP:	-

EXECUTIVE SUMMARY

Effects of two pesticides at sublethal concentrations on fecundity, growth, and development of honeybees *Apis mellifera ligustica* were examined with the feeding method for a three-year period (2006–2008). Material and methods as well as results are only summarized for deltamethrin.
Worker bees (*Apis mellifera ligustica*) were captured from honey and pollen combs in the hive for bioassays. Deltamethrin solutions were prepared in a liquid mixture of 1:1 sucrose:water. Concentrations of deltamethrin were 20.0, 36.0, 64.8, 116.6 and 210.0 mg/L. Control bees were fed a liquid mixture of 1:1 sucrose:water. In each experiment, a treatment included three cages of 20 bees each. The cages were placed in the incubator (30 ±1°C, 60 ±10% relative humidity, darkness). Three cages of 20 bees were used for each concentration of deltamethrin. Experiments were replicated at least three times. Mortality was recorded at 48 h after the feeding.
Experiments to examine the effect of deltamethrin at sublethal dose on fecundity, growth, and development of honeybees commenced on May 15, 2006 and continued on July 20, 2007 and September 1, 2008. New colonies were used each year, a pair of sister queens were used in the same year, and all treatments had five colonies. Each treated colony was fed pesticide solution (400 ml per day) at an estimated 5% lethal concentration (LC5) that was derived from the toxicological tests. The queen was directly fed 5 ml of pesticide solution every 5 d. Pesticides were in a liquid mixture of 1:1 sucrose:water. Control colonies were fed a liquid mixture of 1:1 sucrose:water. All of the colonies were fed for 20 d. The stored honey and syrup were taken out every 3 d to avoid the effects of pesticides concentrated in the stored honey. The queen laid eggs on a new comb within 1 d in the queen excluder. The number of eggs per female per day and their weight was recorded. After hatching, the larva weight was measured within 6 h of when the cells were capped. To check the emergence of adults, the mapped frames were placed in the incubator (34± 1°C, 60 ± 10% relative humidity, darkness) after the cells capped at approximately 9 d.
The LC50 and Lc5 values of deltamethrin in honeybees obtained with oral tests were 60.8 and 21.6 mg/L, respectively.
Daily fecundity. The Tukey's HSD test indicated that and deltamethrin-treated females produced significant fewer ($p \leq 0.05$) eggs than the control in 2006, 2007, and 2008. *Egg weight.* Egg weight indicated significant differences ($p \leq 0.05$) in egg weight between the control and deltamethrin across the three-year period. *Fresh larval weight.* The larval weight of colonies fed deltamethrin was

significantly higher ($p \leq 0.05$) in 2006 and lower ($p \leq 0.05$) in 2008 than the control. *Hatch rate.* Significantly lower hatch rates were found for colonies fed deltamethrin compared with control across the three-year period. The hatch rates for deltamethrin were 62.0, 75.0 and 80.5% in 2006, 2007 and 2008, respectively. *Cap rate.* The cap rate of colonies fed deltamethrin was significantly lower than that of control over the 3-year period (72.0, 93.3 and 85.1% in 2006, 2007 and 2008 respectively). *Emergence rate.* There were no significant differences in the emergence rate between deltamethrin and the control in 2006 and 2008, but there was a significant difference in 2007, and colonies fed deltamethrin had a lower emergence rate compared with the control. *Success rate of development.* The success rate of development in the colonies treated by delamethrin was also lower than the control colonies (40.7, 62.5 and 66.8% in 2006, 2007 and 2008, respectively). *Egg stage.* The duration of the egg stage exposed to deltamethrin was significant longer (Tukey's HSD, $p \leq 0.05$) than that of the control in 2006 and 2007. *Unsealed brood stage.* The unsealed brood stage of the group fed deltamethrin was significantly longer ($p \leq 0.05$) than that of the control in 2007. *Sealed brood stage.* There was also a significant difference ($p \leq 0.05$) in the sealed brood stage between the control and deltamethrin during the three years. *Immature stage.* There was a significant ($p<0.05$) difference in the immature stage between the control and deltamethrin fed colonies during the three years.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin 2.5 EC
Active substance(s):	(S)-alpha-cyano-3-phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropancarboxylate
Adjuvant / Surfactant:	-
Source of test item:	[redacted]
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Sucrose:water (1:1)
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Apis mellifera ligustica*
Cultivar:	-
Source of test species:	-
Age of test organisms at study initiation / Crop growth stage at treatment:	-
Holding conditions prior to test:	Field conditions
Acclimatisation:	-

B. Study design and methods

1.Test procedure

Test system (study type):	Acute oral toxicity (AOT) and chronic oral toxicity (COT)
Duration of study:	AOT: 48 h; COT: 3 years (experiment were commenced on May 15, 2006 and continued on July 20, 2007 and September 1, 2008;

	Food was provided each time period over 20 days)
Treatments:	Deltamethrin and Control (Sucrose:water solution (1:1))
Test concentrations	AOT: 20.0, 36.0, 64.8, 116.6, and 210.0 mg/L; COT: LC5 or AOT
Number of replicates:	AOT: 3 replicates (&3 repetitions); COT: 1 replicate
Individuals per replicate:	AOT: 20 individuals; COT: 5 colonies
Test units (type and size):	AOT: 9 x 9 x 6 cm cages; CTO: Apiaries
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	1:1 sucrose:water solution
Temperature / relative humidity:	AOT: 30 ±1°C / 60 ±10% relative humidity
Photoperiod:	AOT: darkness
Lighting	
pH:	-
Organic matter (C_{org})	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	
Biological parameters measured:	AOT: Mortality; COT: daily fecundity, egg weight, larva weight, hatch rate, cap rate, emergence rate, success rate, egg stage, unsealed brood stage, sealed brood stage, sealed brood stage and immature stage
Measurement frequency:	AOT: mortality were assessed at test end; COT: daily (number of eggs per female)
Statistical analyses:	Probit analysis, one-way analysis of variance (ANOVA), Tukey' s honestly significant difference (HSD) test, χ^2 test

RESULTS

1. Validity criteria:

No validity criteria were mentioned.

3. Biological findings:

The LC50 and Lc5 values of deltamethrin in honeybees obtained with oral tests were 60.8 and 21.6 mg/L, respectively.

Daily fecundity. The Tukey's HSD test indicated that and deltamethrin-treated females produced significant fewer ($p \leq 0.05$) eggs than the control in 2006, 2007, and 2008. *Egg weight.* Egg weight indicated significant differences ($p \leq 0.05$) in egg weight between the control and deltamethrin across the three-year period. *Fresh larval weight.* The larval weight of colonies fed deltamethrin was significantly higher ($p \leq 0.05$) in 2006 and lower ($p \leq 0.05$) in 2008 than the control. *Hatch rate.* Significantly lower hatch rates were found for colonies fed deltamethrin compared with control across the three-year period. The hatch rates for deltamethrin were 62.0, 75.0 and 80.5% in 2006, 2007 and 2008, respectively. *Cap rate.* The cap rate of colonies fed deltamethrin was significantly lower than

that of control over the 3-year period (72.0, 93.3 and 85.1% in 2006, 2007 and 2008 respectively). *Emergence rate.* There were no significant differences in the emergence rate between deltamethrin and the control in 2006 and 2008, but there was a significant difference in 2007, and colonies fed deltamethrin had a lower emergence rate compared with the control. *Success rate of development.* The success rate of development in the colonies treated by delamethrin was also lower than the control colonies (40.7, 62.5 and 66.8% in 2006, 2007 and 2008, respectively). *Egg stage.* The duration of the egg stage exposed to deltamethrin was significant longer (Tukey's HSD, $p \leq 0.05$) than that of the control in 2006 and 2007. *Unsealed brood stage.* The unsealed brood stage of the group fed deltamethrin was significantly longer ($p \leq 0.05$) than that of the control in 2007. *Sealed brood stage.*

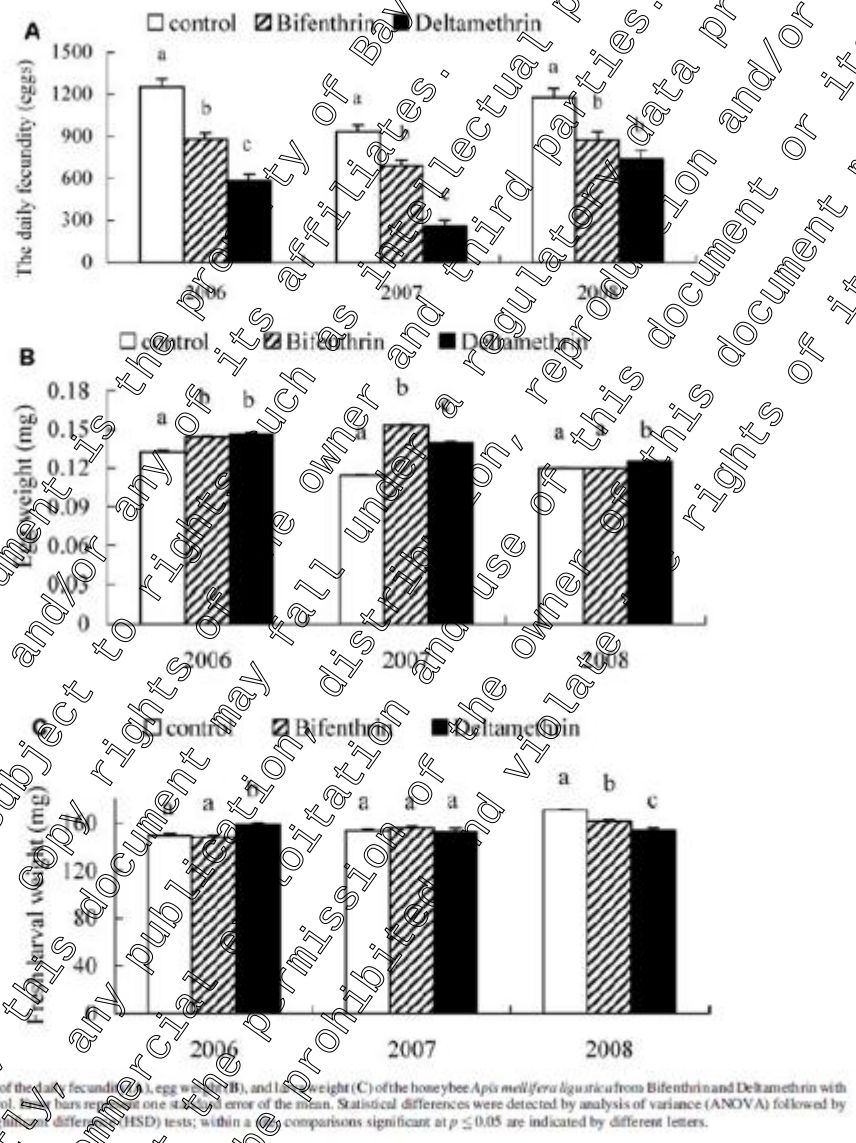


Fig. 1. Comparison of the daily fecundity (A), egg weight (B), and larval weight (C) of the honeybee *Apis mellifera ligustica* from Bifenthrin and Deltamethrin with those from the Control. Error bars represent one standard error of the mean. Statistical differences were detected by analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) tests; within a group comparisons significant at $p \leq 0.05$ are indicated by different letters.

There was also a significant difference ($p \leq 0.05$) in the sealed brood stage between the control and deltamethrin during the three years. *Immature stage.* There was a significant ($p<0.05$) difference in the immature stage between the control and deltamethrin fed colonies during the three years.

Table 1: The median lethal concentration (LC50) and 5% lethal concentration (LC5) values of deltamethrin in *Apis mellifera ligustica*. The LC50 and LC5 values were obtained from the experiments carried out with different bifenthrin and deltamethrin doses after oral application and were calculated by log-probit analysis.

Values represent means of three experiments performed in triplicate. Values in brackets represent 95% confidence limits.

	Oral LC50 (mg/L)	Oral LC5 (mg/L)
Deltamehtrin	60.8 (53.6-69.0)	21.6 (16.5-26.3)

Table 2: The hatch rate, the cap rate, the emergence rate, and the success rate of development of the honeybee *Apis mellifera ligustica* on control, and deltamethrin in 2006, 2007, and 2008. Within a row, samples not included under the same letter (a, b, or c) were significantly different ($p \leq 0.05$) by χ^2 tests.

	Test time	Control	Bifenthrin	Deltamethrin
Hatch rate %	2006	84.0 a	83.1 a	62.0 b
	2007	96.3 a	95.6 a	75.0 b
	2008	88.1 a	71.4 c	80.5 b
Cap rate %	2006	92.1 a	75.9 b	72.0 b
	2007	97.9 a	92.9 b	93.3 a,b
	2008	92.2 a	91.4 a	85.1 b
Emergence rate %	2006	97.4 a	92.1 a	91.0 a
	2007	98.6 a	85.6 b	89.3 b
	2008	97.3 a	92.3 b	97.4 a
Success rate of development %	2006	75.3 a	58.1 b	40.7 c
	2007	93.0 a	76.0 b	62.5 c
	2008	79.0 a	60.2 b	66.8 b

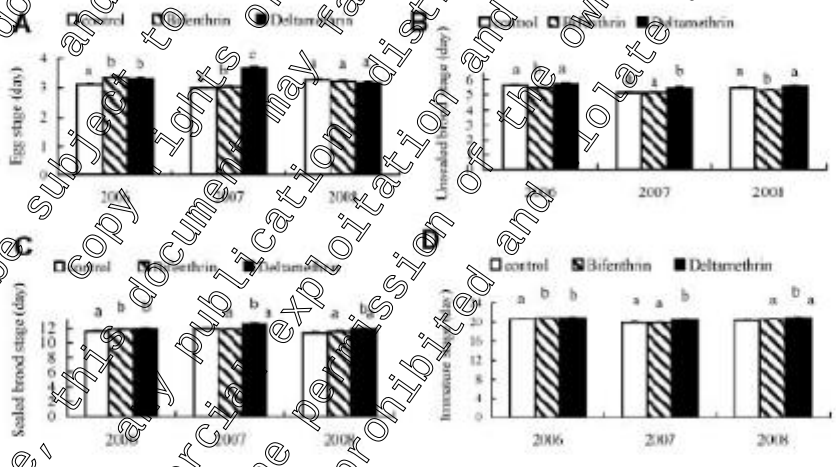


Fig. 2. The development period (mean ± standard error) of the honeybee *Apis mellifera ligustica* on the Control, Bifenthrin and Deltamethrin colonies in 2006, 2007, and 2008. (**A**) The egg stage. (**B**) The unsealed brood stage. (**C**) The sealed brood stage. (**D**) The immature stage. Within a site, bars not included under the same letter (a, b, or c) were significantly different ($p \leq 0.05$) by Tukey's honestly significant difference tests, after an analysis of variance indicated a significant treatment effect.

RESULTS SUMMARY

The 48 h-LC50 and 48 h-LC5 values of deltamethrin in honeybees obtained with oral tests were 60.8 and 21.6 mg/L, respectively.

Comments of the Notifier: The authors applied deltamethrin at a total amount of 8,640 µg to each test colony per day which represents approximately 100 LC_{50} doses per colony per day over 20 days.

On top, they dosed the queen each 5th day with a defined dose. No efforts were made to relate these doses to field-relevant doses. From the physico-chemical properties of deltamethrin it is very unlikely that colonies are exposed to deltamethrin over longer periods and the high lipophilicity makes is very unlikely that nurse bees feed queens with doses applied in this study. Finally, all (semi-)field studies which had been conducted with field-relevant application rates under realistic exposure conditions, i.e. spray treatment of flowering target crops, demonstrated that colonies were not impacted in any endpoint relevant for colony development and performance.

Report:	**KCP 10.3.1.1,1,/11; A.E. Gradish, C.D. Scott-Dupree, A.J. Erwin, G.C. Cutler**
Title:	Lethal and sublethal effects of some insecticides recommended for wild blueberry on the pollinator *Bombus impatiens*
Source:	Can. Entomol. 144: 478–486 (2012)
DOI No:	10.4039/tce.2012.40
Document No:	M-462175-01-1
Guidelines:	None
GLP:	No (Peer-reviewed article)

EXECUTIVE SUMMARY

The susceptibility of common eastern bumble bee (*Bombus impatiens* Cresson) (Hymenoptera: Apidae) to some insecticides used or projected for use in blueberry pest management was investigated. Workers were killed by topical application of deltamethrin and when deltamethrin was ingested via honey solution.
In another experiment, workers were fed one sublethal dose of contaminated honey solution and placed in microcolonies to assess impacts on feeding, life span, and reproduction. The highest concentration of deltamethrin (1.7mga.i./L) reduced feeding. Workers treated with deltamethrin had shortened life spans and produced fewer males.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Decis® 5 EC
Active substance(s): Deltamethrin
Chemical state and description: Not specified
Source of test item: Bayer CropScience, Calgary, Alberta, Canada
Batch number: Not specified
Purity: Not specified
Storage conditions: Not specified
Water solubility: Not specified

2. Test organism(s)

Species: Class "A" colonies of *Bombus impatiens* Cresson
Common name: Common eastern bumble bee
Source of test species: [redacted] Canada)

3. Breeding of test organism(s)

Feeding: A bottle of sugar solution ([redacted], Canada) was included with each colony and provided the bees with nectar substitute *ad libitum*. Honey bee-collected mixed floral pollen pellets were obtained

	from colonies at the University of Guelph Honey Bee Research Facility, ground to a fine powder, and frozen until use. Each colony received about 1 mL of pollen daily.
Observations:	Not specified

B. Study design and methods

1. Contact test:

Test system:	Laboratory test: Test item spray application on adult worker bees
Treatments:	4-5 concentrations causing ~5%–95% mortality were applied; stock solutions were dissolved in deionised water
Control(s):	Control bees were treated with water only.
Number of replicates:	4-5 replicates containing 9-12 sister workers per treatment/control
Pre-treatment:	Bees were anaesthetized in jars with CO_2 for 10–12 seconds and two groups of four to six bees each were then placed dorsal side up in a 5-cm diameter glass Petri dish with a filter paper.
Application technique:	An in-house designed, scaled-down version (1/9th size spray tube) of the Potter[7] spray tower, consisting of a mounted airbrush sprayer that applies an even film of pesticide was used. 1 mL of solution corresponding to each treatment was applied.
Post-treatment:	Bees were transferred to a waxed paper Dixie® cup and received two cotton-plugged, 1-ml plastic flower picks, filled with a 50% honey/water solution as a food source. They were held in the dark at 25±1°C and 30%–40% RH.
Measurements:	Mortality was assessed after 48 hours.
Statistics:	See under "oral test"

2. Oral test:

Test system:	Laboratory test: Feeding of bees with treated honey/water solution
Treatments:	4-5 concentrations causing ~5%–95% mortality were applied; solutions were prepared by dissolving the test item in a 50% honey/deionised water solution
Control(s):	Control bees were provided 50% honey/water solution only
Number of replicates:	4-5 replicates containing 9-12 sister workers per treatment/control
Pre-treatment:	Bees were anaesthetized for 8 seconds and transferred individually into plastic vials. Bees were starved in the dark for 3 hours before use in bioassays.

[7] Potter, C. 1952. An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. Annals of Applied Biology, 39: 1–28.

Application technique: A 25 mL drop of the appropriate solution was placed on the inside vial wall. Bees were allowed to feed for 15 minutes. Bees that did not fully consume the solution were excluded from the experiment.

Post-treatment: Treated bees were transferred individually to waxed paper Dixie® cups and received 50% honey/water solution as a food source. They were maintained in the dark at 25±1°C and 30% RH.

Measurements: Mortality was assessed after 48 hours.

Statistics: Regression line slopes, LC_{50} values, χ^2 values, and 95% fiducial limits were calculated using the probit procedure in SAS 9.1[8]. Hazard quotients were calculated by dividing the manufacturer recommended application rate by its LC_{50} (quotient of <1 suggests the compound is nonhazardous). All tests were performed at a significance level of α=0.05.

3. Sublethal microcolony test:

Test system: Laboratory test: Small groups of worker bees were isolated from colonies and exposed to the test item; the development of new microcolonies was observed

Test concentrations: 17 and 1.7mg a.i./L (formulated product was tested)

Control(s): Yes

Number of replicates: The experiment was repeated three times, with a total of 18 microcolonies per treatment.

Application technique: Done as in the oral test.

Test conditions and duration: Microcolonies were maintained at 25±1°C, 30% RH, and 16:8 h light:dark cycle for 40 days. They were fed with 50% honey/water solution *ad libitum*. Brood rearing was initiated by the introduction of a 2-g pollen ball made of ground pollen, honey and water (5:1:1 ratio). A supplemental 1-g pollen ball was introduced and replaced twice weekly for the entire experiment. Bees, their brood, and pollen were transferred to a new plastic container when faecal contamination occurred, about every 10 days.

Measurements: Daily observations: Number of days to first oviposition and emergence of males, date of worker death, number of ejected larvae and amount of nectar solution consumed (supplemental 1-g pollen balls were weighted)

Statistics: An analysis of variance (ANOVA) using the mixed procedure in SAS 9.1 was performed. A Shapiro–Wilk test confirmed that residuals

[8] SAS Institute. 2005. PROC users manual, version 9.1, 6th ed. SAS Institute, Cary.

were approximately normally distributed. Differences among means were determined with Fisher's least significant difference test (significance level of α=0.05).

2. Chemical analysis

Guideline/protocol: Not specified
Method: Not performed

RESULTS

1. Contact toxicity:

Results for deltamethrin are summarized in table 1.

Table 1:Direct contact toxicity and hazard of formulated deltamethrin to adult worker *Bombus impatiens* 48 hours following spray application.

n	Slope ± SE	LC_{50} (mg a.i./L)	95% FL	x^2	Label rate [mg a.i./L)[A]	Hazard quotient[B]
301	4.4 ± 0.76	346.5	316.8-382.6	5.7	31	0.1

[A] Based on an application volume of 200 L/ha. Where a range of recommended applications rates was given on the formulated insecticide label, a mean rate was used: deltamethrin 6.25 g a.i./ha.
[B] Hazard quotient=insecticide label rate / its LC_{50}. Insecticide with hazard quotient of <1 is considered nonhazardous.
SE, standard error; FL, fiducial limits.

2. Oral toxicity:

Results for deltamethrin are summarized in table 2.

Table 2:Oral toxicity and hazard of formulated deltamethrin to adult worker *Bombus impatiens* 48 hours following ingestion.

n	Slope ± SE	LC_{50} (mg a.i./L)	95% FL	x^2	Label rate [mg a.i./L)[A]	Hazard quotient[B]
260	4.1 ± 0.56	33.8	30.8-37.4	1.5	31	0.9

[A] Based on an application volume of 200 L/ha. Where a range of recommended applications rates was given on the formulated insecticide label, a mean rate was used: deltamethrin 6.25 g a.i./ha.
[B] Hazard quotient=insecticide label rate / its LC_{50}. Insecticide with hazard quotient of <1 is considered nonhazardous.
SE, standard error; FL, fiducial limits.

3. Sublethal microcolony test:

Workers treated with deltamethrin at 1.7 mg a.i./L had significantly reduced survival and produced fewer males compared to the control ($p=0.0001$ and $p=0.0008$, respectively). Results for deltamethrin are summarized in table 3.

Table 3: Sublethal impacts of insecticides on *Bombus impatiens.*

Treatment (mg a.i./L)[A]	Endpoint assessed (mean ± SEM)			
	Life span (days)	Daily nectar consumption (g)	Days to first oviposition	Total males produced
Control (0)	39.5 ± 0.3 ab	2.9 ± 0.1 abc	5.2 ± 0.7 a	9.5 ± 2.0 a
Deltamethrin (1.7)	37.8 ± 0.9 b	2.9 ± 0.1 abc	6.9 ± 0.7 a	6.8 ± 2.0 a
Deltamethrin (17.0)	32.7 ± 1.6 c	2.7 ± 0.1 c	6.3 ± 0.7 a	4.3 ± 2.0 b

[A] Each worker consumed 25mL of honey/water solution mixed with insecticide prior to being placed in a microcolony
[B] Values within columns with the same letter are not significantly different (α=0.05).

RESULTS SUMMARY

48-h toxicity tests with *Bombus impatiens* resulted in LC_{50} values of 346.5 mg deltamethrin/L (contact test) and 33.8 mg deltamethrin/L (oral test), respectively. In the 40-d sublethal microcolony test,

workers treated with deltamethrin at 17 mg a.i./L had significantly reduced survival and produced fewer males compared to the control.

Comments of the Notifier: Gradish *et al.* (2012) examined the effect of deltamethrin on bumble bees (*Bombus impatiens*). From laboratory assays, they calculated an oral LC_{50} of 33.8 mg deltamethrin/L (= 845 µg a.s./bee) which is substantially above the reported contact LC_{50} of 36 µg a.s./bee for *Bombus terrestris*, and suggests that deltamethrin acts primarily via the contact route as described for honey bees. The authors reported significantly reduced survival and reproduction rates of worker bees in micro-colonies which had been fed under laboratory conditions with 1.7 mg a.s./L deltamethrin (≈ 40 µg a.s./bee). No significant reduced survival or reproduction rates were observed for worker bees in micro-colonies which had been fed under laboratory conditions with 1.7 mg a.s./L deltamethrin (≈ 4 µg a.s. /bee). No attempt had been made to relate the applied doses to field-relevant doses. Two tunnel studies with field-relevant application rates (12.5 g a.s./ha) under realistic exposure conditions, i.e. spray treatment of flowering target crops, demonstrated that foraging bumble bees were not impacted in any endpoint investigated.

CP 10.3.1.1.2 Acute contact toxicity to bees

Report:	**KCP 10.3.1.1.2/01, [redacted] (2000)**
Title:	Contact toxicity (LD_{50}) to honey bees (*Apis mellifera* L.) Deltamethrin oil in water emulsion 15 g/L Code: AE F032640 00 EW01 B103
Document No:	M-198509-01-1 (Rep. No. CW00/032)
Guidelines:	EPPO 170
GLP:	Yes

Material and Methods:

Groups of fifty worker honey bees, *Apis mellifera*, were treated by topical application with the dose rates of 0.4; 0.8; 1; 2 and 4 µg test item per bee. After 24, 48 and 72 hours the numbers of dead bees in each cage were assessed.

Findings:

	AE F032640 00 EW01 B103
Time (hours)	**LD_{50} (95 % fiducial limits) µg product per bee**
24	0.943 (0.851 – 1.055)
48	0.921 (0.826 – 1.026)
72	0.871 (0.781 – 0.972)

*****

Report:	**KCP 10.3.1.1.2/02, [redacted] (2000)**
Title:	Contact toxicity (LD_{50}) to honey bees (*Apis mellifera* L.) Deltamethrin emulsifiable concentrate 15 g/L
Document No:	M-198885-01-1 (Rep. No.: CW00/030)
Guidelines:	EPPO Guideline No.170
GLP:	yes

Material and Methods:

In this laboratory study the contact toxicity of the insecticide AE F032640 00 EC02 A804 to the worker honey bee was determined. The study was designed to comply with EPPO Guideline No.170

and under GLP. Groups of fifty worker honey bees, *Apis mellifera*, were treated by topical application with the dose rates of 0.2; 0.4; 0.8; 1.0 and 2.0 µg AE F032640 00 EC02 A804 per bee. After 24, 48 and 72 hours the number of dead bees in each cage were assessed.

Findings:

	AE F032640 00 EC02 A804
Time (hours)	LD_{50} (95% fiducial limits) µg product per bee
24	1.368 (1.371 – 1.894)
48	1.481 (1.307 – 1.743)
72	1.494

*****

Report:	**KCP 10.3.1.1.2/03, [redacted] (2000)**
Title:	Contact toxicity (LD_{50}) to honey bees (*Apis mellifera* L.) Deltamethrin emulsifiable concentrate 100 g/L
Document No:	M-198786-01-1 (Rep. No.: CW00/031)
Guidelines:	EPPO Guideline No.170
GLP:	yes

Material and methods:
Groups of fifty worker honey bees, *Apis mellifera*, were treated by topical application with the dose rates of 0.04; 0.08; 0.1; 0.2 and 0.4 µg test item per bee. After 24, 48 and 72 hours the numbers of dead bees in each cage were assessed.

Findings:

AE F032640 00 EC11 A308	
Time (hours)	**LD_{50} (95 % fiducial limits) µg product per bee**
24	0.288 (0.249 – 0.342)
48	0.277 (0.242 – 0.326)
72	0.264 (0.221 – 0.332)

Conclusion:
The contact LD_{50} was set at 0.264 µg product/bee after 72 h of exposure.

*****

Report:	**KCP 10.3.1.1.2/04, [redacted] (1994)**
Title:	Determination of the contact toxicity by topical application of Decis WG2 (Hoe 032640 00 EG06 A101) to the honey bee *Apis mellifera* L.
Document No:	M-139791-01-1 (Rep. No.: CW94/030E)
Guidelines:	EPPO Guideline No.170
GLP:	yes

Material and Methods:
The contact toxicity of AE F032640 00 EG06 A101 following topical application was investigated in the laboratory. A single dose of 1 µl of the test substance was applied to each bee with the aid of a microapplicator. Inspections took place 24, 48 and 72 hours after treatment. The LD_{50} was calculated

by means of probit analysis.

Findings:

	AE F032640 00 EG06 A101
Time (hours)	LD_{50} (95% fiducial limits) µg a.i. per bee
24	0.074 (0.065 – 0.085)
48	0.068 (0.060 – 0.078)
72	0.064 (0.056 – 0.073)

*****

Supplemental information from the literature

Report:	**KCP 10.3.1.1.2/05; Carvalho, S. M.; Carvalho, G. A.; Carvalho, C. F.; Bueno Filho, J. S. S.; Baptista, A. P. M.. (2009)**
Title:	Toxicity of acaricides/insecticides for citrus crop to the africanized honeybee apis mellifera l., 1758 (hymenoptera: apidae) original title: toxicidade de acaricidas/inseticidas empregados na citricultura para a abelha africanizada apis mellifera l., 1758 (hymenoptera: apidae)
Source:	Arquivos do Instituto Biologico, 76, 4, p. 597-605.
DOI No: Document No:	- M-461215-01-2
Guidelines:	-
GLP:	-

EXECUTIVE SUMMARY

This study was performed to assess the actions of chemical products on *A. mellifera* adults when applied by spraying, ingestion of contaminated food and exposure to treated surfaces. Material and methods as well as results are summarized for deltamethrin only.

Deltamethrin (25EC) was tested at a concentration of 50ml deltamethrin per100 L water. Distilled water or pure food was used for the control treatments. The methods used in our experiments were chosen with the aim of obtaining results for various modes of exposure of the bees to the pesticides, knowing that this can occur by direct contact during spraying, settling on treated surfaces and ingestion of contaminated food. The tests were performed on an entirely randomized basis with 10 repetitions per treatment, with each experimental unit being formed by 10 adult bees kept in a climatized room at 25 ± 2°C, r.h. 70± 10% and 12-hour photo phase.

Spraying of products on *A. mellifera:* One hundred bees per treatment were placed in a Petri dish and sprayed with the products by means of a Potter tower at a rate of 1.5 ± 0.5 µL/cm2 at 15 psi. They were then transferred to PVC cages (15 cm diameter x 10 cm height) Sugar paste[9] was given as food and cotton wool soaked with distilled water was placed on the tulle in the upper part of the cage.

Provision of product-contaminated sugar paste to *A. mellifera:* The sugar paste was prepared with 50 g of icing sugar and 10 ml of honey. The amount of pesticide to be incorporated was determined according to the diet volume and the pre-established dose. After placing 10 bees in each PVC cage, the sugar paste contaminated with each of the pesticides was placed on the tulle in the upper part of the

[9] WAHL, O. Le nourrissement. In: CHAUVIN, R. (Ed.). Traité de bologie de l'Abeille. Paris: Masson et Cie, 1968. Tome IV, p.162-180.

cage together with cotton wool imbibed with distilled water.

Residual effect of pesticides on *A. mellifera* using a contaminated glass surface: The inside surfaces of the Petri dishes (10 cm diameter x 2 cm height) were sprayed with the pesticides by means of a Potter tower at an application rate of 1.5 ± 0.5 µL/cm^2 at 15 psi. After evaporation of the excess of each pesticide, the food packed in a plastic lid of 2 cm diameter was placed inside the chambers, followed by the anaesthetized bees.

Residual effect of pesticides on *A. mellifera* using contaminated citrus leaves: Tangerine leaves (Citrus reticulate Blanco cv. Ponkan) collected from a plant exempt from pesticide treatment were immersed for 5 seconds in the chemical mixtures of each treatment. To eliminate the excess fluid, the leaves were placed for about 3 hours in a ventilated place in the dark. After drying, four treated leaves were placed in each chamber set. Food packed in a plastic lid was placed in the base of each chamber, followed by the previously anaesthetized bees.
The assessment times were standardized in all the experiments, ending when the control treatment mortality was 20% or more (EPA-OPPTS 1996). The assessments were made at 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60 and 72 hours.
The experiments were performed in duplicate in a factorial scheme (9 treatments x 4 application methods) with measurements repeated in time, whereby the data obtained underwent survival analysis using the Survival packet[10] compiled by Software R® (2008).
The respective 50% lethal times (LT50) were also calculated[11,12]

Spraying of products on *A. mellifera*: Deltamethrin showed low toxicity wioth an LT_{50} of 178.57 hours.

Provision of product-contaminated sugar paste to *A. mellifera*: Deltamethrin caused mortality that increased with increasing exposure time to reach 67% after 72 hours, with an LT50 of 64.65 hours.

Residual effect of pesticides on *A. mellifera* using a contaminated glass surface: Deltamethrin was toxic with an LT50 of 42.91 hours and a final mortality of 64% (48 h).

Residual effect of pesticides on *A. mellifera* using contaminated citrus leaves: Deltamethrin was toxic to bees, with mortalities of 100% respectively and a mean LT50 of 27.74 hours.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Deltamethrin 25 EC
Active substance(s): Deltamethrin
Adjuvant / Surfactant: -
Source of test item: -

[10] THERNEAU, T.; LUMLEY, T. Survival analysis, including penalised likelihood. Package version 2.43-1, 78p. 2008.
[11] MONCHARMONT, F.X.D; DECOURTYE, A; HANTIER, C.H; PONS, O.; PHAM-DELEGUE, M. Statistical analysis of honeybee survival after chronic exposure to insecticides. Environmental Toxicology and Chemistry, v.22, p.3088-3094, 2003.
[12] COLOSIMO, E.A.; GIOLO, S. R. Análise de sobrevivência aplicada. São Paulo: Edgar Blucher, 2006. 392p.

Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	Africanized bees (*Apis mellifera*)
Cultivar:	
Source of test species:	[redacted]
Age of test organisms at study initiation /	adult
Crop growth stage at treatment:	
Holding conditions prior to test:	
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Spraying; consumption of contaminated sugar paste, residual effect of contaminated glass plate and citrus leaves
Duration of study:	Until control treatment was 20% or more; (up to 72 h)
Treatments:	Control (distilled water); Deltamethrin
Test concentrations	50 ml/100L
Number of replicates:	10
Individuals per replicate:	10
Test conditions:	25 ± 2°C, r.h. 70± 10% and 12-hour photo phase
Test units (type and size):	Spraying and consumption of contaminated sugar paste: PVC cages (15 cm diameter x 10 cm height); Residual effect: Petri dishes of 10 cm diameter x 2 cm height
Application / device / nozzles:	Potter tower (1.5 ± 0.5 µL/cm2 at 15 psi)
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Spraying and consumption of contaminated sugar paste: PVC cages; Residual effect: contaminated glass surface or contaminated tangerine leaves
Temperature / relative humidity:	25 ± 2°C, r.h. 70± 10%
Photoperiod:	12-hour photo phase
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60 and 72 hours.
Statistical analyses:	survival analysis using the Survival packet[10]; Weibull distribution (Sgrilo 1982)

RESULTS

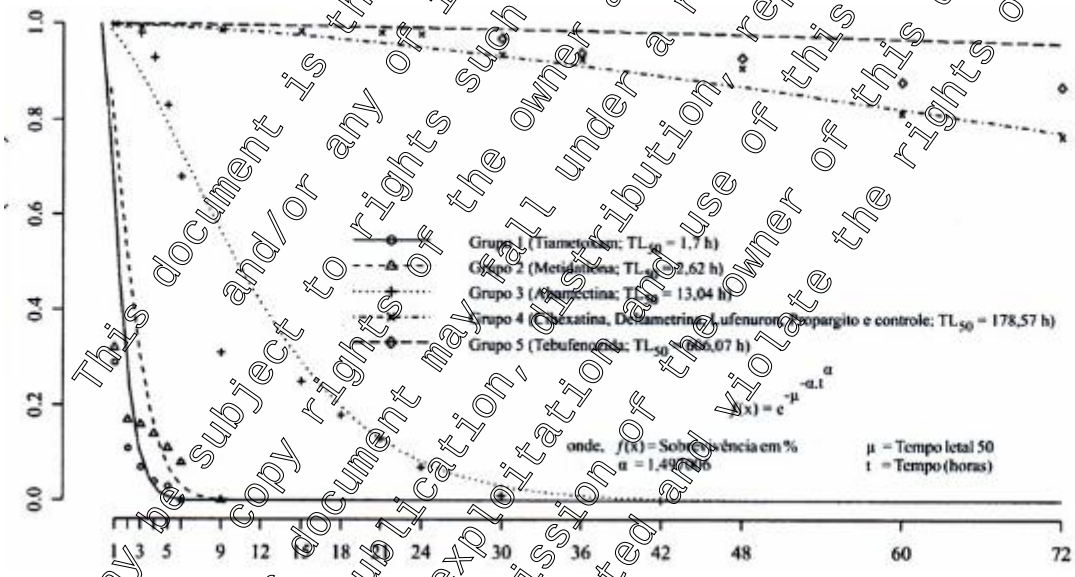
1. Validity criteria:

No validity criteria were stated. However, the assessment times were standardized in all the experiments, ending when the control treatment mortality was 20% or more (Epa-Oppts 1996).

2. Biological findings:

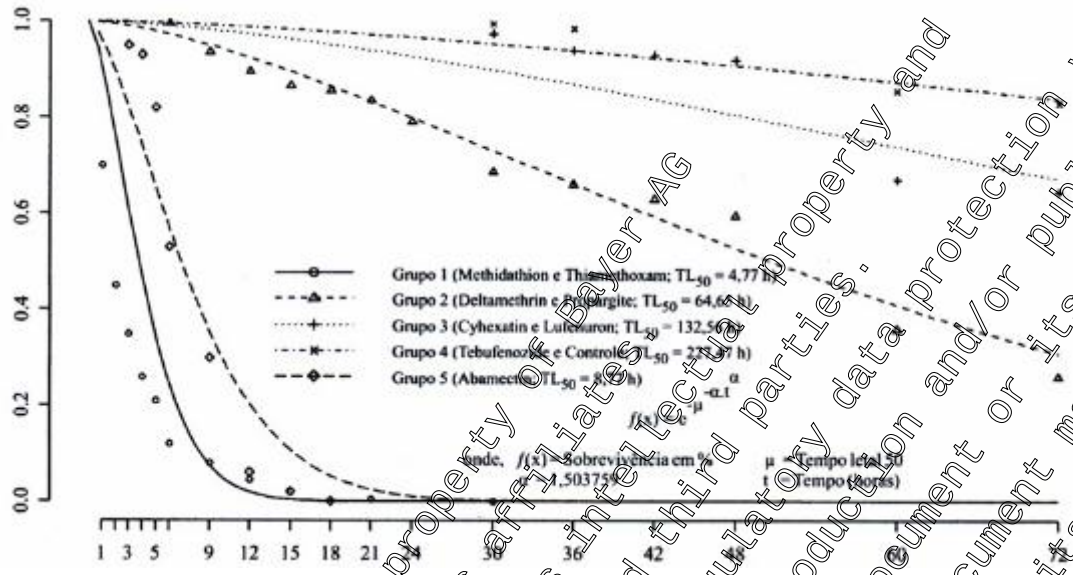
Spraying of products on *A. mellifera:* Deltamethrin showed low toxicity wioth an LT_{50} of 178.57 hours.

Figure 1: Survival (%) of Apis mellifera workers after spraying with pesticides: lethal times (LT50) in hours.



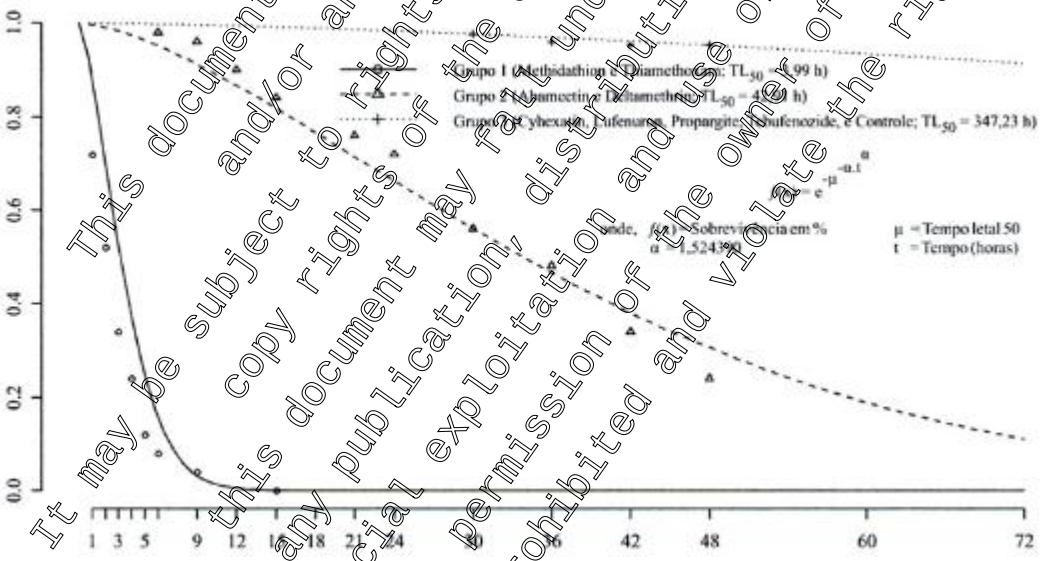
Provision of product-contaminated sugar paste to *A. mellifera:* Deltamethrin caused mortality that increased with increasing exposure time to reach 67% after 72 hours, with an LT50 of 64.65 hours.

Figure 2: Survival (%) of Apis mellifera workers after providing Cândi paste contaminated with pesticides: lethal times (LT50) in hours.



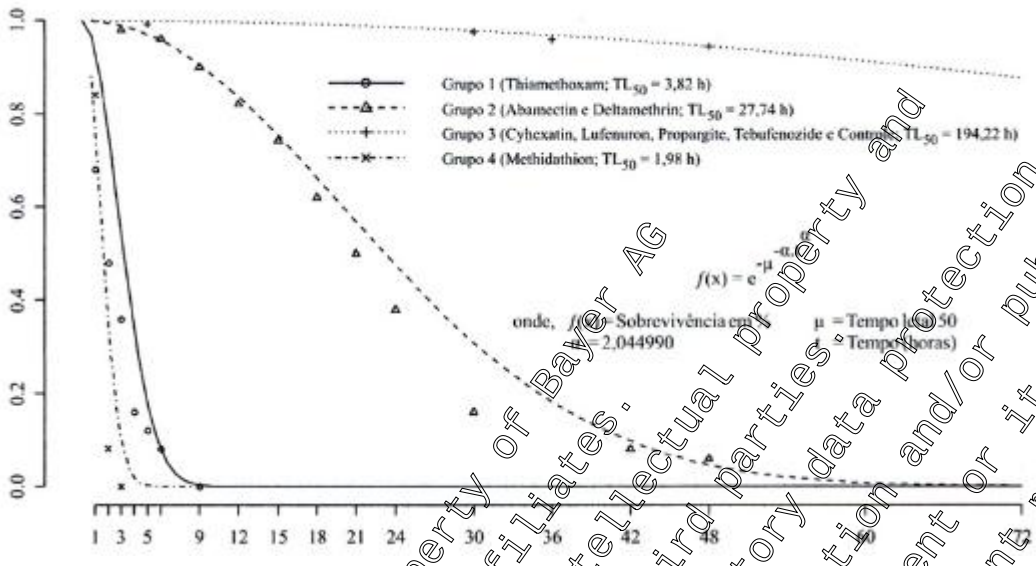
Residual effect of pesticides on *A. mellifera* using a contaminated glass surface: Deltamethrin was toxic with an LT50 of 42.91 hours and a final mortality of 64% (48 h).

Figure 3: Survival (%) of *Apis mellifera* workers after contact with a glass surface contaminated with pesticides: lethal times (LT_{50}) in hours.



Residual effect of pesticides on *A. mellifera* using contaminated citrus leaves: Deltamethrin was toxic to bees, with mortalities of 100% respectively and a mean LT50 of 27.74 hours.

Figure 4: Survival (%) of *Apis mellifera* workers after contact with citrus leaves contaminated with pesticides: lethal times (LT_{50}) in hours.



RESULTS SUMMARY

Spraying of products on *A. mellifera*: Deltamethrin showed low toxicity wioth an LT_{50} of 178.57 hours.

Provision of product-contaminated sugar paste to *A. mellifera*: Deltamethrin caused mortality that increased with increasing exposure time to reach 67% after 72 hours, with an LT50 of 64.65 hours.

Residual effect of pesticides on *A. mellifera* using a contaminated glass surface: Deltamethrin was toxic with an LT50 of 42.91 hours and a final mortality of 64% (48 h).

Residual effect of pesticides on *A. mellifera* using contaminated citrus leaves: Deltamethrin was toxic to bees, with mortalities of 100% respectively and a mean LT50 of 27.74 hours.

Comment of the Notifier: Individual honey bees which were dosed under laboratory / indoor conditions suffered from various adverse effects in a dose-dependent manner. However, the ability of large honey bee colonies to absorb impacts from stressors and continue to grow makes extrapolation from effects observed in a laboratory on individual bees to effects of relevance at the colony level in the field extremely difficult. In many cases, acute risks of lethal or sub-lethal effects for individual bees observed in the laboratory may have no consequences whatever for colonies in the field. In summary, testing on individual bees does not allow for an evaluation of potential impacts on the entire colony: only on the basis of field and monitoring studies is it possible to determine whether a particular stressor gives rise to a colony level impact.

Report:	**KCP 10.3.1.1.2/06; Carvalho, S. M.; Belzunces, L. P.; Carvalho, G. A.; Brunet, J.-L.; Badiou-Beneteau, A. (2013).**
Title:	Enzymatic biomarkers as tools to assess environmental quality: a case study of exposure of the honeybee *Apis mellifera* to insecticides
Source:	Environmental Toxicology and Chemistry, Vol. 32, No. 9, pp. 2117–2124
Document No.:	M-464768-01-1
Guidelines:	European and Mediterranean Plant Protection Organization guideline 170 Deviations: higher requirement for control mortality (<5%)
GLP:	No

EXECUTIVE SUMMARY

The present study was intended to evaluate the responses of enzymes in the honeybee *Apis mellifera* after exposure to deltamethrin and its use as biomarkers. The responses of acetylcholinesterase (AChE), carboxylesterases (CaEs-1–3), glutathione-S-transferase (GST), catalase (CAT), and alkaline phosphatase (ALP) were assessed. For the enzyme involved in the defense against oxidative stress, deltamethrin induced no CAT activity. However, exposure to deltamethrin induced slight effects and modulated only CaE^{-1} and CaE^{-2}, with opposite effects.

After determination of the median lethal doses (LD_{50}), honeybees were exposed at doses of 5.07 ng/bee and 2.53 ng/bee for deltamethrin, (equivalent to 1/10th [$LD_{50/10}$] and 1/20th [$LD_{50/20}$] of the LD_{50}, respectively). The LD_{50} was calculated as 50.65 ng deltamethrin/bee after 48 h.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	deltamethrin
Active substance(s):	deltamethrin
Adjuvant / Surfactant:	Not reported
Source of test item:	Sigma-Aldrich
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test organism(s)

Species:	Honeybees (*A. mellifera*)
Cultivar:	Not relevant
Source of test species:	reared at the experimental apiary of the Institut National de la [redacted] France
Age of test organisms at study initiation :	Workers
Holding conditions prior to test:	put in cages (10.5 cm _ 7.5 cm _ 11.5 cm), fed ad libitum with candy paste and water, and kept at 25 +/- 2°C and 60 +/- 10% relative humidity.
Acclimatisation:	gathered on the day before the experiment

B. Study design and methods

1. Test procedure

Test system (study type):	Acute laboratory toxicity study
Duration of study:	48h
Treatments:	Once at beginning
Test concentrations	2 controls; not reported for acute toxicity test; 5.07 ng/bee and 2.53 ng/bee of deltamethrin for further bioassays
Number of replicates:	Not reported
Individuals per replicate:	30 individuals
Test units (type and size):	plastic cages (10.5 cm x 7.5 cm x 11.5 cm) in groups of 30 individuals
Application / device / nozzles:	between 8:00 AM and 10:00 AM, the honeybees were mildly anesthetized with CO2, and 1 mL of insecticide solution

containing the appropriate dose was applied to the dorsal thorax by means of a Hamilton microsyringe coupled with an automatic dispenser

2. Environmental conditions

Test medium:	Plastic cages
Temperature / relative humidity:	25 +/- 2°C / 60%
Photoperiod:	Not reported
food	candy and water ad libitum

3. Observations and measurements:

Analytical parameters measured:	none
Biological parameters measured:	mortality
Measurement frequency:	After 24 and 48 h
Statistical analyses:	Dose-response assay (drc package)

RESULTS AND DISCUSSION

1. Biological findings:

The LD_{50} was calculated as 50.65 ng deltamethrin/be after 48 h with a confidence interval of 43.33-57.97.

From the LD50 values of each insecticide, the sublethal doses LD50/20 and LD50/10 were determined and used in the exposure assays.

Table 1: Acute toxicity (median lethal dose [LD_{50}]) of deltamethrin to *Apis mellifera*

	Purity (%)	LD_{50} (ng/bee at 48h)	95% CI	X^2	df	$LD_{50/20}$	$LD_{50/10}$
deltamethrin	99.8	50.65	43.33 – 57.97	21.88	15	2.53	5.07

CONCLUSION

The LD_{50} was calculated as 50.65 ng deltamethrin/be after 48 h.

Comment of the Notifier: Carvalho *et al.* (2013) exposed honey bees under laboratory conditions to lethal and sub-lethal doses of deltamethrin via the contact route of exposure. The 48h-LD_{50} of technical grade deltamethrin was found to be 0.051 ng a.s./bee, which is in line with the regulatory database. Sub-lethal dose of deltamethrin (LD_{50}/20 and LD_{50}/10) were found to have a short-term knock-down effect with a full recovery of the bees after 1 - 2 hours after exposure. The investigated sub-lethal doses of deltamethrin resulted in a modulation of some of the investigated biomarkers without inducing mortality.

CP 10.3.1.2 Chronic toxicity to bees

A chronic study was performed with the active substance deltamethrin ([redacted] 2014, M-477250-01-1) and is included in the MCA document (see MCA 8.3.1.2/01).

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

A honey bee brood semi-field study (OECD GD 75) has been conducted with the EW 15-formulation ([redacted], 2014; M-477316-01-1) and is included under CP 10.3.1.5, below.

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.

Supplemental information from the literature

Report:	**KCP 10.3.1.4/01; Song, H.; Zhou, T.; Wang, Q.; Dai, P.; Luo, Q.; Xu, S.; Wu, Y. (2011).**
Title:	Effects of sublethal doses of insecticides on olfactory sensitivity of honeybee (Apis mellifera ligustica)
Source:	Yingyong Kunchong Xuebao, 48, 3, p. 611-615
DOI No: Document No:	- M-462163-01-2
Guidelines:	-
GLP:	-

EXECUTIVE SUMMARY

The authors observed the proboscis extension reflex of the honeybee as a means of studying the effects of sub-lethal doses of deltamethrin and imidacloprid on honeybee olfactory sensitivity, to provide an evidentiary basis for the reasonable use of insecticides and to protect the honeybee. Material and methods as well as results are summarized for deltamethrin only.

The test used Italian honeybee Apis mellifera ligustica L. worker bees obtained from the Experimental Apiary of the Honeybee Protection and Biological Safety Laboratory of the Institute for Apicultural Research of the Chinese Academy of Agricultural Sciences; the insecticides used were 0.6% deltamethrin miscible oil preparation (Jiangsu Province Yixing City Yizhou Chemical Products). Young worker bees (18 days old) were randomly selected for the bioassay. Step 1: After the worker bee was starved for 2 hours, it was removed from the incubator (35°C and 67-70 % relative humidity), and outside the incubator a glass wand dipped in water was used to approach the antenna of the worker bee. Proboscis extension was observed and recorded. The same method was used for each concentration in sequence at 0.1%, 0.3%, 1%, 3%, 10% and 30% sucrose solutions to stimulate the worker bee. The water and each concentration of sucrose solution were used to stimulate at time intervals of 3 minutes and the proboscis extension response was recorded. Step 2: After 0.5 hours, each bee was fed 10 µL 30% sucrose solution containing sub-lethal doses of deltamethrin (5 and 10 ng) and then placed back into the incubator. Step 3: After 2 hours, step 1 was repeated, and the proboscis extension response of the honeybees to water and to each concentration of sucrose solution were recorded.

Sublethal doses of deltamethrin reduced the worker bees' sensitivity to water. Furthermore, it was discovered through $\chi 2$ testing that after oral feeding of 5 ng deltamethrin, the proboscis extension response rate of the worker bees when stimulated by a concentration of 0.1% sucrose solution was significantly reduced ($P < 0.05$), while its proboscis extension response rates to stimulation at other concentrations of sucrose did not change significantly. After oral feeding of 10 ng deltamethrin, the decline in the proboscis extension response rate of the honeybee to stimulation by 0.1% sucrose

solution was significant ($P < 0.05$), while the decline in proboscis extension response rate to stimulation by 1% sucrose solution reached extremely high significance ($P < 0.01$).

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	0.6% deltamethrin miscible oil preparation
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	Jiangsu Province Yixing City Yizhou Chemical Products
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Apis mellifera ligustica* L.
Cultivar:	-
Source of test species:	Experimental Apiary of the Honeybee Protection and Biological Safety Laboratory of the Institute for Apicultural Research of the Chinese Academy of Agricultural Sciences
Age of test organisms at study initiation:	18 days old worker bees
Crop growth stage at treatment:	-
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Proboscis extension reflex (PER)
Duration of study:	-
Treatments:	Delamethrin and control (no deltamethrin)
Test concentrations:	5 and 10 ng/µl in 30% sugar solution
Number of replicates:	3
Individuals per replicate:	30; However: 5ng: N=31; 10 ng: N=33
Test units (type and size):	-
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	30% sucrose solution
Temperature / relative humidity:	In incubator: 35°C and 65-70% relative humidity
Photoperiod:	-
Lighting	-
pH:	-
Organic matter (C_{org}):	-

$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Proboscis extension reflex to water and 1%, 0.3%, 1%, 3%, 10% and 30% sucrose solutions
Measurement frequency:	3 minutes intervalls
Statistical analyses:	$\chi 2$ testing

RESULTS

1. Validity criteria:

No validity criteria were stated.

2. Biological findings:

Sublethal doses of deltamethrin reduced the worker bees' sensitivity to water. Furthermore, it was discovered through $\chi 2$ testing that after oral feeding of 5 ng deltamethrin, the proboscis extension response rate of the worker bees when stimulated by a concentration of 0.1% sucrose solution was significantly reduced ($P < 0.05$), while its proboscis extension response rates to stimulation at other concentrations of sucrose did not change significantly. After oral feeding of 10 ng deltamethrin, the decline in the proboscis extension response rate of the honeybee to stimulation by 0.1% sucrose solution was significant ($P < 0.05$), while the decline in proboscis extension response rate to stimulation by 1% sucrose solution reached extremely high significance ($P < 0.01$).

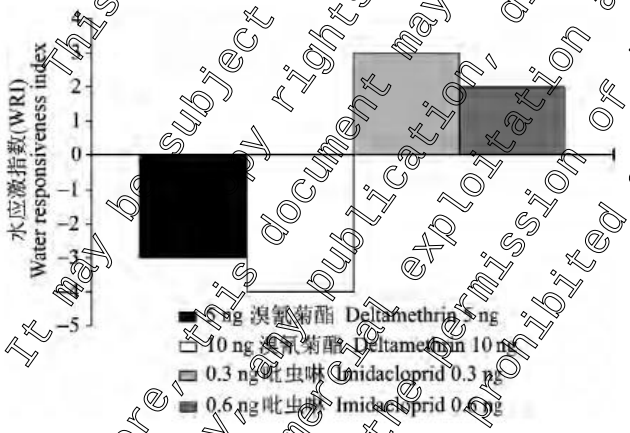


图 1 溴氰菊酯和吡虫啉对工蜂水应激指数的影响

Fig. 1 Effects of deltamethrin and imidacloprid on the WRIs of *Apis mellifera ligustica*

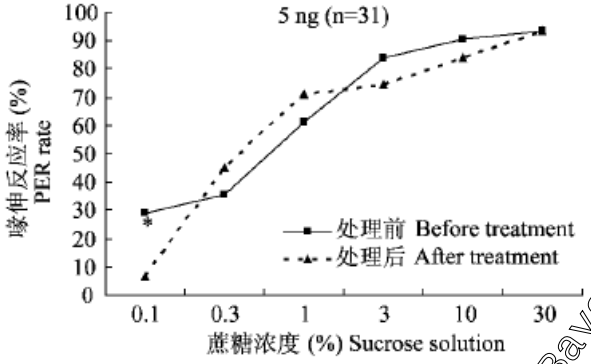


图 2　5ng 溴氰菊酯对工蜂喙伸反应的影响

Fig. 2　Effects of 5ng deltamethrin on the PER of *Apis mellifera ligustica*

* 表示经 χ^2 检验差异显著（$P < 0.05$），** 表示差异极显著（$P < 0.01$）。下图同。

* Significant difference at the 0.05 level by χ^2 test; ** Significant difference at the 0.01 level by χ^2 test. The same below.

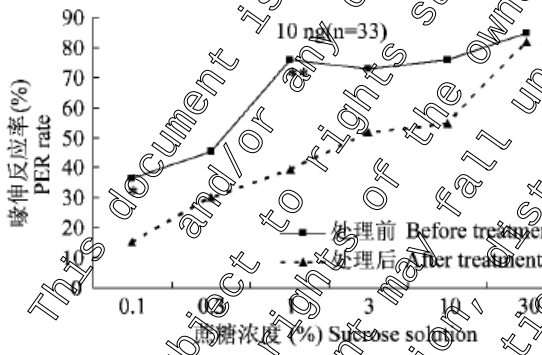


图 3　10 ng 溴氰菊酯对工蜂喙伸反应的影响

Fig. 3　Effects of 10 ng deltamethrin on the PER of *Apis mellifera ligustica*

RESULTS SUMMARY

Sublethal doses of deltamethrin reduced the worker bees' sensitivity to water and sub-lethal doses of deltamethrin caused a decline in honeybee sensitivity to sucrose solution and this effect became more notable as the pesticide concentrations increased.

Comment of the Notifier: Individual honey bees which were dosed under laboratory / indoor conditions suffered from various adverse effects in a dose-dependent manner. However, the ability of large honey bee colonies to absorb impacts from stressors and continue to grow makes extrapolation from effects observed in a laboratory on individual bees to effects of relevance at the colony level in the field extremely difficult. In many cases, acute risks of lethal or sub-lethal effects for individual bees observed in the laboratory may have no consequences whatever for colonies in the field. In summary, testing on individual bees does not allow for an evaluation of potential impacts on the entire

colony: only on the basis of field and monitoring studies is it possible to determine whether a particular stressor gives rise to a colony level impact.

CP 10.3.1.5 Cage and tunnel tests

During the evaluation of the AIR dossier for Deltamethrin the RMS UK noted that a number of study summaries on the bee tunnel studies for deltamethrin do not contain sufficient information. The RMS UK requested to submit revised study summaries for a number of reports summarized below. The study summaries were written according to a master template provided by the RMS UK and include daily tabulated mortality rates per replicate.

Report:	**KCP 10.3.1.5/01, [redacted]:2001**
Title:	Tunnel test - Acute and short term effects of AE F032640 00 EW01 B106, applied on white mustard on honey bees (*Apis mellifera* L.)
Document No:	M-204260-01-1 (Rep. No.: S01AVB.879VO44)
Guidelines:	EPPO 170, (1992), CEB 129
GLP:	yes

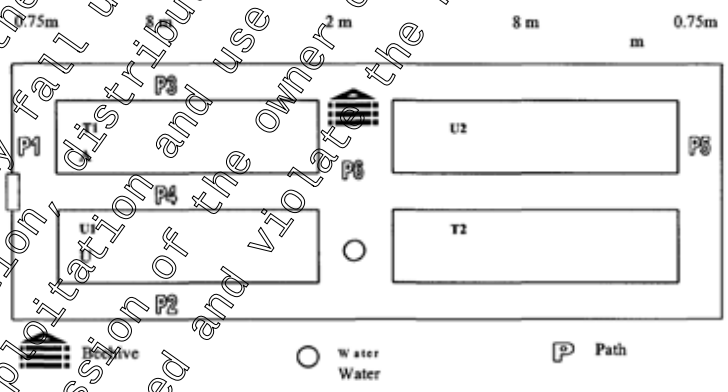
~~**Material and Methods:**~~
~~Bees were confined within tunnels on flowering white mustard fields. After an acclimatization phase of seven days, application was performed during bee flight. The control was treated with water, the test item was applied at a rate of 0.5 L/ha, as a non-toxic standard, Zolone Flo was used at a rate of 1.2 L/ha. The test substance treatment was twice replicated, control and standard once. Endpoints assessed were mortality, foraging and flight activity, storage of honey and pollen, behavior, and brood development.~~

~~**Findings:**~~
~~Mortality and flight activity in the test substance treatment were similar as in the non-toxic standard. Foraging activity was not or only very slightly affected by the test substance treatment on the treated as well as on the refuge areas in the tunnels. Likewise, no effects on the behaviour were detected. Brood development was not affected by the test substance treatment as well.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EW 15 (Decis 15 EW, AE F032640 00 EW01 B106) content of a.s. deltamethrin: 1.51 % w/w (15 g/L nominal)
Batch number:	TA 161/99PM
Reference item:	Zolone Flo SC 500 (500 g a.s./L nominal, analysed content: 499 g a.s./L)
Test organism:	Honey bees (*Apis mellifera L.*) The used hives were single box colonies (type DADANT) with 10 frames, 10.000 bees and one queen at test start. Queens were obtained by grafting. and colonies (consisting of Caucasian bees) were homogeneous as possible. At the beginning of the test, the oldest worker honeybees were a maximum of 3 months old.

	Furthermore the following criteria for each colony were guaranteed: • 4 frames containing brood combs • 2 frames for reserves • 4 frames were kept empty for free space An empty new frame of known weight was introduced in each hive just prior to their introduction into the tunnels.
Source:	[redacted] (Supplier)
Crop:	White mustard, *Sinapis alba*, cultivar: Silenda, sown June 21, 2001; Bees were released at the BBCH stage 62 (20 % of opened flowers)
Test location:	Merainville in central France (Beauce area), Cheramy's Farm, Thiville, France. The apiary part was conducted in St Ambroix.
Test unit:	The study was composed of 4 tunnels. Each tunnel was made of metal arches covered by plastic netting. The ground area covered was approximately 136.5 m^2 (19.5 m x 7 m) and the height was approximately 3 m at the highest point of the arch. The tunnels were placed with their long side perpendicular to the seed row. All tunnels were positioned with the same orientation regarding compass bearing. The diagram below shows the area within the tunnels, which were divided into four areas (T1, T2 and U1, U2) of 16 m^2 (8 x2 m) each. Areas T1 and T2 were areas that have received treatments while U1 and U2 were refuge zones not receiving any treatment.



T1 and T2: treated areas
U1 and U2: refuge zones not receiving any treatment

In each tunnel one hive was placed and assignment to tunnels was randomised.

Application rates:	Control (C): Mineral water ("Christal Roc") Treatment rate 1: 0.5 L/ha (7.5 g a.s./ha) during foraging activity Treatment rate 2: 0.5 L/ha (7.5 g a.s./ha) during foraging activity Reference rate (R): 1.2 L/ha (600 g a.s./ha) Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).

	The spray volume was 300 L/ha in all treatment groups. The sprayer was calibrated before use on the day of application. The deviation reached a maximum of 3.12 %.
Data sampling:	Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.
Data analysis:	Linear regression analysis using STAT-ITCF was done to compare the mortality of the bees during the acclimatisation phase and the mortality of the bees during the exposure period.
Deviations from the study plan:	One deviation was recorded in the study report. This deviation had no impact on the study because only the batch number was changed. The product was still Decis 15 EW.

Climatic conditions during the experiment:

The environmental parameters recorded were within the normal range for the region. No dramatic weather conditions such as storms or violent winds occurred during the study period. The environmental conditions are shown in the table below.

Table 1: Field conditions

Date	Temperature Mini °C	Temperature Maxi °C	Relative air humidity % Mini	Relative air humidity % Maxi	Rainfall mm
01.08.01	17.1	32.0	40	92	-
02.08.01	17.7	28.0	52	91	9.0
03.08.01	13.0	25.3	44	99	3.2
04.08.01	13.2	22.4	47	100	-
05.08.01	15.5	25.7	53	100	-
06.08.01	17.2	23.0	71	100	-
07.08.01	14.3	22.0	79	100	17.4
08.08.01	14.3	22.1	52	96	0.6
09.08.01	9.3	21.9	46	99	1.2
10.08.01	7.6	22.1	35	99	-
11.08.01	8.0	24.6	33	100	-
12.08.01	10.8	27.0	35	100	-
13.08.01	12.5	29.3	33	100	0.4
14.08.01	14.8	33.4	26	100	-
15.08.01	19.9	32.2	37	100	23.4
16.08.01	14.4	41.4	40	100	0.2
17.08.01	14.6	30.5	95	100	-
18.08.01	16.3	29.8	75	100	1.8
19.08.01	13.6	23.5	50	98	3.8
20.08.01	11.8	23.8	55	100	0.2
21.08.01	12.6	27.6	41	100	-
22.08.01	14.5	29.7	35	100	-

[1] Data coming from Météorologie nationale Jailans (28), the nearest station.

Pesticide history of the field site:

Previous pesticide history of the test site is listed in the table below.

Table 2: Pesticide history of the field site

Year	Crop	Pesticides			
		Herbicides Name(a.i), rate	Fungicides Name(a.i), rate	Insecticides Name(a.i), rate	Other Name(a.i), rate
2000	Winter wheat (Thésée)	**Célio+Agral** (clodinafop+cloquintocet) 0.6+1l/ha	**Unix** (cyprodinil) 1kg/ha **Marathon** (cyproconazol + chlorothalonil) 2l/ha	**Gaucho blé** (imidacloprid+bitertanol+anthraquinone 0.4l/100kg seeds **Karate** (lambda-cyalothrine) 0.125l/ha	**Terpal** (mepiquat+éthéphon) 2l/ha
1999	Corn (Anjou 285)	**Gesaprime auto** (atrazine) 2l+0.8l/ha **Mikado** (sulcotrione) 0.7l/ha		**Gaucho** (imidacloprid) 0.07l/50000 seeds **Karate** (lambda-cyalothrine) 0.125l/ha	
1998	Winter wheat (Cézanne)	**Starane 200 +Allié** (fluroxypyr+metsulfuron-méthyle) 1L+0.015kgl/ha **Chardex** (dopyralid+fluroxypyr+méco-prop-p) 2L/ha	**Alto 100SL** (cyproconazol) 0.8l/ha **Caramba** (metconazole) 0.8l/ha	**Karate xpert** (lambda-cyalothrine) 0.125l/ha	**Cycocel C5** (chlormequat chloride+choline chloride) 2L/ha

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EW 15 on the honeybee, *Apis mellifera,* under forced exposure conditions.

This study included four exposure groups (tunnels) each: one tap-water treated control group, two test-item groups and one reference item group. In all exposure groups, the crop was sprayed 7 days after set-up of the hives in the tunnels (Acclimatisation phase) at BBCH 62 (20% flowering), during honeybees actively foraging on the crop under confined conditions. The honeybees remained 17 days in the tunnels.

Depending on the weather conditions the observations were conducted for a 10-day period following a 7-day adaptation period of the hives to the confinement. At the end of this 10-day period, symptoms of toxicity (mortality, behaviour, etc.) were no more clearly observed in each tunnel, the exposure phase of the study was stopped and beehives returned to the apiary.

The assessments of the number of any dead bees were performed in each tunnel, each morning during both the adaptation period of the hives and thereafter until the end of the test. For each tunnel, these daily assessments was performed commencing August 10 in the morning (not possible before because of the rain) and subsequent daily assessment was conducted at approximately the same time for each tunnel each day. This daily assessment was not performed August 18th and 19th because of bad weather conditions. During each assessment all dead bees were collected in the 6 paths and in the dead bee trap (the bees collected from each of the path areas 1 to 5 were pooled).

The assessments of the foraging activity were performed only on those days when the weather is such that honeybees are likely to be foraging. Counting was done over the whole of the zone by assessing the number of bees passing an area of 60cm wide, whilst walking at a normal speed down the side of the zone. The foraging activity of the bees was expressed as the number of bees observed separately within each zone on each assessment. After the adaptation period of the beehive, the foraging activity was evaluated twice a day at regular intervals (starting around 10 a.m. in the morning and 3 p.m. in the afternoon). In addition, the day of treatment foraging activity is assessed around 15 minutes before treatment and around 15 minutes and 1 hour after each treatment.

Before the foraging assessments were performed within each tunnel the number of bees leaving and entering the hive were recorded (when possible) over a five-minute period. In order to avoid any mistake,

counting of bees entering the hive was done for a 150 seconds period, then counting for bees leaving the hive for a 150 seconds period with another second sequence of 5 minutes.

Duration of flower visits was assessed during same time number of the assessment bees leaving and entering the hive. This was performed by recording the time (in seconds) that 15 different bees forage over 15 different attractive plants (This was done for 15 bees with a maximum time of 90 seconds in order not to delay the following assessments). The plants chosen for this assessment were chosen without conscious bias from those available within each tunnel.

Behaviour of bees was observed during assessment of bee mortality, foraging activity and control of the colony. Bees were observed for abnormalities like aggressiveness, intensive flying without landing on the crop, moribund or severely affected bees, bee accumulation at the beehive entrance, trembling, bees no longer producing pollen balls, etc..

The following endpoints were assessed:

- Cumulative number of dead bees before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Number of foraging bees per zone (T1, T2 and U1, U2) and number of bees/m² in each tunnel before as well as after the applications in the control, the test item groups and the reference item group, respectively.
- Number of bees leaving and entering the beehives in the control, the test item groups and the reference item group, respectively
- Duration of lower visits in the control, the test item groups and the reference item group, respectively
- Behaviour of the bees during assessments in the control, the test item groups and the reference item group, respectively
- Control of the colony with the following criteria examined: weight of the empty frame introduced into the centre of the hive, for both sides of each frame the percentage frame surface area containing honey, for both sides of each frame the percentage frame surface area containing pollen, for both sides of each frame the percentage frame surface area containing eggs, for both sides of each frame the percentage surface area of brood (young and old larvae) in each frame and % of capped and uncapped alveolus as well as the health of the queen.

Dates of Work: 6th August to 11th October 2001

Findings:

Honey Bee Mortality:

A summary of the daily mortality (cumulated dead bees) is shown in following table.

Table 3: Cumulated dead bees during the study period (only males and worker-bees considered)

Date	Tunnel No. 1 Deltamenthrin EW 15 @ 7.5 g a.s./ha	Tunnel No. 2 Deltamenthrin EW 15 @ 7.5 g a.s./ha	Tunnel No. 4 Water	Tunnel No. 3 Dimethoate @ 600 g a.s./ha

	Males	Workers	Total	Males	Workers	Total	Males	Workers	Total	Males	Workers	Total
10.08.01 3DBT	0	1200	1200	3	6006	6009	0	2894	2894	0	1866	1866
11.08.01 2DBT	0	1720	1720	3	7211	7214	4	3656	3660	0	2329	2329
12.08.01 1DBT	1	2281	2282	111	8519	8630	15	4326	4341	0	3596	3596
13.06.01 0DBT	3	2532	2535	190	8918	9108	29	4865	4894	2	4375	4377
14.06.01 1DAT	3	2796	2799	207	9553	9760	30	4997	5027	2	4899	4901
15.06.01 2DAT	4	2986	2990	232	10073	10305	35	5107	5142	8	5374	5382
16.06.01 3DAT	5	2989	2994	232	10076	10308	38	5114	5152	8	5383	5391
17.06.01 4DAT	7	3014	3021	246	10216	10462	39	5142	5181	8	5526	5534
20.06.01 7DAT	9	3037	3046	259	10323	10582	40	5161	5201	9	5627	5636
21.06.01 8DAT	9	3065	3074	259	10449	10708	40	5169	5209	9	5682	5691
22.06.01 9DAT	9	3083	3092	261	10767	11028	40	5178	5218	9	5794	5803

DBT: days before treatment
DAT: days after treatment

The effect of Deltamethrin EW 15 on bee mortality was similar to the effect on the bee mortality of the non-toxic standard Zolone Flo. This was true for the tunnel No. 1 or No. 2 even if the daily mortality was higher in tunnel No. 2 (the daily mortality in tunnel No. 2 was higher before and after the treatment).

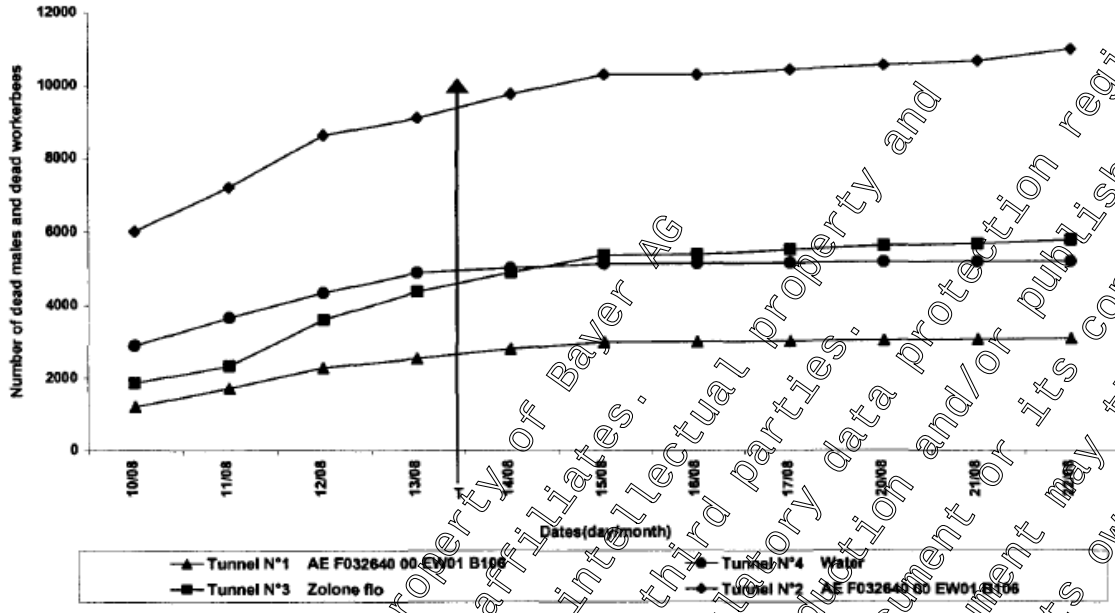


Figure 1: Cumulative mortality of bees in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) at 7.5 g a.s./ha and Zolone Flo.
(T= treatment)

Honey Bee Foraging activity

Deltamethrin EW 15 had no or very limited effect on the foraging activity in the days following the treatment on both treated and refuges areas.

Table 4: Number of bees foraging in the treated zones (T1, T2) in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) at 7.5 g a.s./ha and Zolone Flo.

Assessment date (Day/month/hour)	Number of bees/m² (means)			
	Tunnel N°4 Water	Tunnel N°1 AE F032640 00 EW01 B106	Tunnel N°2 AE F032640 00 EW01 B106	Tunnel N°3 Zolone Flo
10/08 - 14h05-14h24	1,41	2,00	1,75	0,97
11/08 - 14h34-14h53	2,97	2,41	2,56	1,91
11/08 - 16h23-16h43	2,03	1,69	2,16	0,91
12/08 - 13h42-14h00	3,56	2,56	3,06	1,81
12/08 - 16h25-16h40	1,50	1,41	1,31	0,53
13/08 - 10h45-11h04	5,72	5,72	3,56	3,59
13/08 - 11h30-12h06	3,41	3,31	2,66	2,34
13/08 - 12h07-12h34	2,53	1,41	0,97	2,37
13/08 - 13h05-13h36	3,13	2,38	1,28	1,91
13/08 - 16h38-16h45	0,97	1,09	0,78	0,53
14/08 - 11h32-11h48	4,44	3,69	4,53	2,06
15/08 - 11h13-11h29	5,53	4,22	4,03	3,41
15/08 - 13h45-14h00	2,47	2,32	2,56	2,00
16/08 - 13h55-14h20	5,72	3,75	3,69	2,44
16/08 - 16h58-17h13	0,84	0,63	1,09	0,53
17/08 - 11h16-11h36	9,56	6,91	5,75	4,81
17/08 - 14h04-14h23	6,13	3,66	3,78	3,00
20/08 - 11h28-11h50	7,47	6,84	5,50	3,38
20/08 - 14h16-14h35	3,53	4,88	4,28	3,34
21/08 - 10h51-11h11	8,75	8,25	6,66	5,50
21/08 - 14h00-14h40	6,75	7,94	6,19	5,03
22/08 - 10h39-10h57	9,13	9,00	5,25	5,91
22/08 - 13h42-14h00	5,97	4,47	3,31	3,63

Treatments [redacted]

Table 5: Number of bees foraging in the refuge zones (U1, U2) in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) at 7.5 g a.s./ha and Zolone Flo.

Assessment date (Day/month/hour)	Number of bees/m² (means)			
	Tunnel N°4 Water	Tunnel N°1 AE F032640 00 EW01 B106	Tunnel N°2 AE F032640 00 EW01 B106	Tunnel N°3 Zolone Flo
10/08 - 14h05-14h24	1,50	1,56	1,31	0,44
11/08 - 14h34-14h53	3,00	2,38	3,50	1,16
11/08 - 16h23-16h43	1,91	1,47	2,31	0,88
12/08 - 13h42-14h00	3,25	2,50	3,84	1,47
12/08 - 16h25-16h40	1,44	1,06	0,94	0,59
13/08 - 10h45-11h04	5,59	4,09	3,41	2,78
13/08 - 11h30-12h06	3,09	2,72	2,63	1,97
13/08 - 12h07-12h34	1,97	1,38	2,19	1,31
13/08 - 13h05-13h36	2,75	1,81	1,69	1,28
13/08 - 16h38-16h45	0,72	0,75	0,47	0,38
14/08 - 11h32-11h48	4,03	3,69	4,38	1,78
15/08 - 11h13-11h29	4,75	3,16	4,09	2,50
15/08 - 13h45-14h00	2,78	1,72	2,53	1,91
16/08 - 13h55-14h20	4,66	3,28	3,91	1,72
16/08 - 16h58-17h13	0,72	0,69	1,09	0,81
17/08 - 11h16-11h36	8,53	5,84	6,41	3,94
17/08 - 14h04-14h23	4,38	3,09	4,31	2,69
20/08 - 11h28-11h50	4,94	5,75	5,47	2,88
20/08 - 14h16-14h35	4,31	4,59	5,31	2,91
21/08 - 10h51-11h11	7,63	7,41	7,31	5,16
21/08 - 14h00-14h40	7,44	7,84	6,66	5,66
22/08 - 10h39-10h57	8,34	8,91	4,81	5,56
22/08 - 13h42-14h00	5,16	5,28	3,97	4,16

Treatments

Bees leaving and entering the beehives

Deltamethrin EW 15 did not show any effect different from the non-toxic standard Zolone Flo on the number of bees leaving and entering the beehive just after the treatment.

Table 6: Bees leaving and entering the beehives in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) at 7.5 g a.s./ha and Zolone Flo.

Assessment date (Day/month/hour)	Number of bees/m² (means)							
	Tunnel N°4 Water		Tunnel N°1 AE F032640 00 EW01 B106		Tunnel N°2 AE F032640 00 EW01 B106		Tunnel N°3 Zolone Flo	
	Leaving	Entering	Leaving	Entering	Leaving	Entering	Leaving	Entering
10/08 - 14h05-14h24	83,00	101,00	126,00	139,00	192,00	206,00	41,00	59,00
13/08 - 16h38-16h45	134,00	167,00	91,00	101,00	121,00	145,00	183,00	227,00
14/08 - 11h32-11h48	37,00	35,00	44,00	37,00	23,00	44,00	87,00	107,00
15/08 - 11h13-11h29	40,00	42,00	41,00	36,00	37,00	31,00	41,00	43,00
15/08 - 13h45-14h00	38,00	35,00	17,00	14,00	27,00	29,00	19,00	25,00
16/08 - 13h55-14h20	20,00	19,00	35,00	37,00	44,00	40,00	21,00	25,00
16/08 - 16h58-17h13	11,00	13,00	18,00	21,00	17,00	14,00	11,00	14,00
17/08 - 11h16-11h38	66,00	70,00	51,00	48,00	61,00	55,00	34,00	29,00
17/08 - 14h04-14h23	54,00	42,00	26,00	23,00	35,00	31,00	20,00	18,00
20/08 - 11h28-11h50	36,00	31,00	43,00	46,00	40,00	36,00	21,00	17,00
20/08 - 14h16-14h35	39,00	38,00	39,00	34,00	47,00	49,00	20,00	19,00
21/08 - 10h51-11h11	66,00	58,00	63,00	57,00	61,00	53,00	42,00	36,00
21/08 - 14h00-14h40	51,00	46,00	53,00	58,00	55,00	59,00	42,00	45,00
22/08 - 10h39-10h57	67,00	58,00	67,00	63,00	37,00	41,00	54,00	49,00
22/08 - 13h42-14h00	56,00	54,00	49,00	47,00	46,00	42,00	40,00	42,00

Treatments [redacted]

Duration of flower visits

The results did not show any effect of Deltamethrin EW 15 on the duration of flower visits.

Table 7: Duration of flower visits (based on 15 bees) in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) at 7.5 g a.s./ha and Zolone Flo.

Assessment date (Day/month/hour)	Total in seconds (based on 15 bees)			
	Tunnel N° Water	Tunnel N°1 AE F032640 00 EW01 B106	Tunnel N°2 AE F032640 00 EW01 B106	Tunnel N°3 Zolone Flo
10/08 - 14h05-14h24	70,90	79,09	104,80	233,04
13/08 - 16h38-16h45	62,39	149,29	173,33	34,39
14/08 - 11h32-11h48	58,30	70,05	84,01	68,52
15/08 - 11h13-11h29	64,51	78,11	86,03	104,64
15/08 - 13h45-14h00	73,05	76,59	101,63	94,12
16/08 - 13h55-14h20	107,76	121,97	123,52	82,60
16/08 - 16h58-17h13	175,22	90,53	188,43	140,28
17/08 - 11h16-11h38	73,92	67,31	64,47	89,56
17/08 - 14h04-14h23	48,54	115,05	94,57	88,63
20/08 - 11h28-11h50	67,92	37,64	80,16	131,23
20/08 - 14h16-14h35	83,53	133,17	115,34	159,19
21/08 - 10h51-11h11	74,81	83,20	68,50	93,30
21/08 - 14h00-14h40	55,59	57,15	51,41	62,86
22/08 - 10h39-10h57	76,55	60,86	45,18	102,82
22/08 - 13h42-14h00	51,03	66,72	103,41	68,50

Treatments
[redacted]

Behaviour of the bees

Deltamethrin EW 15 had no effect on the bee behaviour in the days following the treatment.

Control of the colony

Reserves and brood were reduced during the study in all the tunnels except for tunnel No. 4. Deltamethrin EW 15 had no negative effect on the control of the colony and this at each date of assessment.

Assessments of the control of the colony are listed in the following tables.

Table 8: Control of the colony exposed to water treated white mustard (Tunnel No. 4)
NR = Not relevant, T = Treatment

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	1100	1400
% frame surface area containing honey	Side a	10	70	20	20	15	15	20	15	20	15	15	20	0	0
	Side b	20	60	15	20	15	15	10	15	15	15	15	20	0	20
% frame surface area containing pollen	Side a	0	0	5	0	0	0	0	0	0	0	0	5	0	0
	Side b	0	0	10	10	0	5	0	10	0	0	0	5	0	0
% frame surface area containing eggs	Side a	NR	NR	0	NR	30	5	NR	15	10	NR	0	NR	NR	NR
	Side b	NR	NR	0	NR	30	5	0	25	10	NR	NR	NR	NR	NR
% surface area of brood	Side a	0	0	70	0	80	20	90	30	80	15	80	0	0	0
	Side b	0	0	70	10	80	20	90	30	80	20	80	5	0	0
% capped alveolus	Side a	NR	NR	30	NR	5	80	100	0	10	100	90	NR	NR	NR
	Side b	NR	NR	80	100	5	75	95	0	50	100	100	100	NR	NR
% uncapped alveolus	Side a	NR	NR	30	NR	95	10	0	100	90	0	10	NR	NR	NR
	Side b	NR	NR	10	0	95	10	5	100	50	0	0	0	NR	NR
		T		T		T		T		T		T		T	

Table 9: Control of the colony exposed to Deltamethrin EW 15 treated white mustard (Tunnel No. 1) at 7.5 g a.s./ha

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	650	1000
% frame surface area containing honey	Side a	50	10	20	10	15	10	15	15	15	10	20	15	0	0
	Side b	60	10	15	10	15	10	15	15	15	10	20	10	0	30
% frame surface area containing pollen	Side a	0	0	10	0	0	2	0	0	0	0	50	0	0	0
	Side b	10	0	15	2	0	5	0	2	0	0	50	0	0	0
% frame surface area containing eggs	Side a	NR	NR	0	NR	NR	NR	0	NR	20	NR	NR	NR	NR	NR
	Side b	NR	NR	0	NR	NR	NR	0	NR	10	NR	NR	NR	NR	NR
% surface area of brood	Side a	0	0	70	0	80	5	80	0	80	0	0	0	0	0
	Side b	0	0	70	0	70	5	80	0	80	0	0	0	0	0
% capped alveolus	Side a	NR	NR	30	NR	100	100	90	NR	20	NR	NR	NR	NR	NR
	Side b	NR	NR	5	NR	100	100	80	NR	15	NR	NR	NR	NR	NR
% uncapped alveolus	Side a	NR	NR	70	NR	0	0	10	NR	80	NR	NR	NR	NR	NR
	Side b	NR	NR	95	NR	0	0	20	NR	80	NR	NR	NR	NR	NR
		T		T		T		T		T		T		T	

NR = Not relevant, T = Treatment

Table 10: Control of the colony exposed to Deltamethrin EW 15 treated white mustard (Tunnel No. 2) at 7.5 g a.s./ha

Oservations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.8	06. 8	22.6
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	1200	1300
% frame surface area containing honey	Side a	30	20	15	15	15	10	15	15	15	10	10	5	0	15
	Side b	20	15	20	10	15	10	15	15	15	5	15	5	30	20
% frame surface area containing pollen	Side a	10	0	0	0	0	0	0	0	0	0	0	0	0	0
	Side b	30	5	0	0	0	0	0	0	0	0	0	5	0	0
% frame surface area containing eggs	Side a	NR	NR	0	0	0	NR	0	0	40	0	15	NR	NR	NR
	Side b	NR	NR	0	0	0	0	0	0	20	NR	0	NR	NR	NR
% surface area of brood	Side a	0	0	80	10	80	0	80	50	80	10	80	10	0	0
	Side b	0	0	80	5	80	20	80	70	80	5	80	10	0	0
% capped alveolus	Side a	NR	NR	90	95	70	NR	70	50	10	0	20	100	NR	NR
	Side b	NR	NR	90	97	80	90	80	80	20	100	80	100	NR	NR
% uncapped alveolus	Side a	NR	NR	10	15	30	NR	30	50	80	100	80	0	NR	NR
	Side b	NR	NR	10	3	20	10	20	20	80	0	5	0	NR	NR
		T		T		T		T		T		T		T	

NR = Not relevant, T = Treatment

Conclusion:

The effect of Deltamethrin EW 15 on bee mortality was similar to the effect on the bee mortality of the non-toxic standard Zolone Flo. This was true for tunnel No. 1 or No. 2 even if the daily mortality was higher in tunnel No. 2 (the daily mortality in tunnel No. 2 was higher before and after the treatment). Deltamethrin EW 15 had no or very limited effect on the foraging activity in the days following the treatment on both treated and refuges areas. Deltamethrin EW 15 had not shown any effect different from the non-toxic standard Zolone Flo on the number of bees leaving and entering the beehive just after the treatment. The results did not show any effect of Deltamethrin EW 15 on the duration of flower visits. Deltamethrin EW 15 had no effect on the bee behaviour in the days following the treatment. Deltamethrin EW 15 had no negative effect on the control of the colony and this at each date of assessment.

*****

Report:	**KCP 10.3.1.5/02, [redacted]; 2005**
Title:	Tunnel test - Acute and short-term effects of Deltamethrin EW 15 and Thiacloprid & Deltamethrin QD 110 applied on white mustard or phacelia, on honey bees (*Apis mellifera* L)
Document No:	M-272845-01-1 (Rep. No.: S05BAB.DELVO16)
Guidelines:	EPPO Bulletin No. 170
GLP:	yes

~~**Material and Methods:**~~

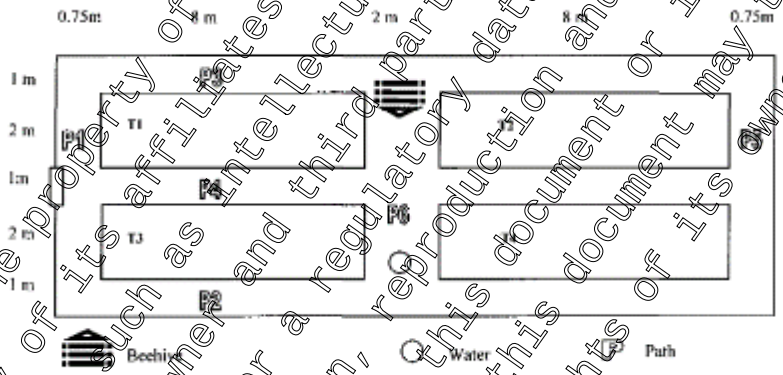
~~Bees were confined within tunnels on flowering phacelia, fields. After an acclimatization phase of seven days, application was performed during foraging activity or without foraging activity. The control was treated with water, the test item was applied at a rate of 0.5 L/ha (Deltamethrin EW 15), as~~

~~a toxic standard, Dimethoate 400EC was used at a rate of 1.0 L/ha. The test substance treatment, control and standard were replicated once. Endpoints assessed were mortality, foraging activity, storage of food, honey and pollen and brood development.~~

~~**Findings:**~~
~~Deltamethrin EW 15 applied during foraging activity showed a relatively slight toxic effect the day of application. Nevertheless, this toxic effect was, by far, lower than the toxic standard toxicity. When applied out of any foraging activity period, Deltamethrin EW 15 showed no effect the day following the application and a very limited effect two days later. The Itox values were quite low in both cases, the level of mortality stayed at a very acceptable level taking into account the high level of mortality in the toxic standard tunnel. Deltamethrin EW 15 had no or a very limited impact on foraging activity except in the hours or the day following the application when applied during foraging activity. This impact was somewhat longer, one day more, when Deltamethrin EW 15 was applied out of any foraging activity. Reserve reduction (honey and pollen) and brood reduction were nil in the water (control) tunnel. This was the same thing for Deltamethrin EW 15 whatever the type of application.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EW 15 content of a.s.: deltamethrin: 16.08 g/L
Batch number:	OP240778
Reference item:	Dimethoate 400EC (batch no.: 75180, 400.0 g a.s./L nominal, analysed content: 400.9 g a.s./L)
Test organism:	Honey bees (*Apis mellifera*) The used hives were single box colonies (type DADANT 10 frames) with 10 frames, one queen and about 10000 to 15000 bees per hive at test start. Queens were obtained by grafting (1 month). A maiden queen (Carnica) was introduced in a hive containing brood combs (with mature pupa) and honeycombs. Afterwards, the hive was placed in a mix apiary close to beehives containing local drones. After one month it was assumed that a homogeneous colony composed of Carniolan bees was obtained. For the conduct of the study, those hives were chosen which passed a confinement test (avoiding hyper-sensitivity). Oldest worker honeybees were a maximum of 3 month old at test initiation and only healthy bee colonies were used (veterinarian certificate). Following criteria for each colony were guaranteed: • 4 frames containing eggs, larvae and capped cells • 2 frames containing honey and pollen • 4 frames were kept empty for free space Additionally, an empty new frame of known weight was introduced in each hive prior to their introduction into the tunnels.
Source:	[redacted] (Supplier)
Crop:	Phacelia, 5.9 kg/ha, sown April 30, 2005; Growth stage at test: BBCH 62

Test location:	[redacted] France ([redacted]). The apiary part was conducted in [redacted].
Test unit:	Each tunnel covered a ground area of 136.5 m^2 (19.5 m × 7 m) with a height of approximately 3 m. The tunnel tent frames were covered with light plastic netting. Furthermore, each tunnel was divided into four areas (T1, T2, T3 and T4) of 16 m^2 (8 m × 2 m) each, containing the crop. Areas T1 and T2 were areas that have received treatments while U1 and U2 were refuge zones not receiving any treatment. The diagrammatic representation of a tunnel is shown in the following figure:  T1 and T2: treated areas U1 and U2: refuge zones not receiving any treatment Areas were separated from each other by a path for observation. In each tunnel one hive was placed and assignment to tunnels was randomised
Application rates:	Control (C): mineral water (Cristalline) Treatment rate 1: 7.5 g a.s./ha during foraging activity Treatment rate 2: 7.5 g a.s./ha out of foraging activity Reference rate (R): 400 g a.s./ha Every treatment comprised of one replicate (i.e. 1 tunnel per treatment). The spray volume was 200 L/ha in all treatment groups. The sprayer was calibrated before use. The deviation reached a maximum of 6.25 %.
Data sampling:	Data for mortality, foraging activity, duration of flower visits, behaviour of the bees and data of the colony were assessed.
Data analysis:	No statistical analysis was used.
Deviations from the study plan:	No deviation to the study protocol.

Climatic conditions during the experiment:

The environmental parameters recorded were within the normal range for the region No dramatic weather conditions such as storms or violent winds occurred during study period. The environmental conditions are shown in table below.

Table 11: Environmental field conditions

Date	Temperature Mini °C	Temperature Maxi °C	Relative[1] air humidity % Mini	Relative[1] air humidity % Maxi	Rainfall mm
01.07.05	10.4	20.9	59	97	-
02.07.05	16.7	26.2	52	96	
03.07.05	13.7	30.6	35	98	
04.07.05	12.7	20.9	38	96	20.0
05.07.05	7.5	21.7	40	97	5.0
06.07.05	11.8	20.5	57	97	-
07.07.05	11.8	18.9	50	90	
08.07.05	12.6	19.9	44	91	-
09.07.05	11.8	20.6	43	96	7.5
10.07.05	13.2	24.3	31	96	1.5
11.07.05	16.1	29.2	28	91	
12.07.05	15.9	29.3	40	98	-
13.07.05	16.2	29.6	33	97	-
14.07.05	15.8	31.0	31	89	-

[1] Data coming from Météorologie nationale, Chateaudun (28) the nearest station

07.07.05: Treatment

Pesticide history of the field site:

Previous pesticide history of the test site and neighbour plot is listed in the table below.

Table 2: Maintanance and pesticide history of the test site and the neighbour plot

Year (Site)	Crop	Pesticides			
		Herbicides Name (a.i.), rate	Fungicides Name (a.i.), rate	Insecticides Name (a.i.), rate	Other Name (a.i.), rate
2004 (test site)	Spring barley	Megaplus (imazamethabenz-methyl+ pendimethelin) 3 L/ha Illoxan CE (diclofop-methyl) 1.4 L/ha	Opera (pyraclostrobin+ epoxiconazol), 0.5 L/ha Amistar (azoxystrobin), 0.5 L/ha	-	Terpal (mepiquat-chlorure+ethephon), 1.5 L/ha Cybéle (ethephon), 0.3 L/ha
2005 (neighbour plot)	Corn	Dual Gold Safeneur (s-metolachlore+ benoxacor) 2.1 L/ha Milado (sulcotrione) 1.5 L/ha	-	Karate zeon (lambda-cyhalothrin), 0.125 L/ha	-

The aim of the study was to evaluate the acute and short-term effects of a foliar treatment Deltamethrin EW 15, applied on phacelia during foraging activity or without foraging activity, on the mortality, behaviour and foraging activity of honey bees, *Apis mellifera L.* under semi-field conditions (tunnels).

This study included four exposure groups (tunnels) with one replicate (tunnel) each: one water treated control group, two test-item groups and one reference item group. In all exposure groups, crop was sprayed 4 days after set-up of the hives in the tunnels (Acclimatisation phase) at BBCH 62 (flowering), during honeybees actively foraging (afternoon) or later (evening) when bees were not foraging on the crop. The honeybees remained all in all 12 days in the tunnels.

Depending on the weather conditions the observations were conducted for a 8 day period following a 4-day adaptation period of the hives to the confinement. At the end of this 8-day period the exposure phase of the study was stopped and beehives returned to the apiary on July 14th.

The assessments of the number of any dead bees were performed in each tunnel, each morning during both the adaptation period of the hives and thereafter until the end of the test. For each tunnel, these daily assessments were performed commencing July 05 in the morning and subsequent daily assessment was conducted at approximately the same time for each tunnel each day. During each assessment all dead bees were collected in the 6 paths and in the dead bee trap (the bees collected from each of the path areas 1 to 5 were pooled).

The assessments of the foraging activity were performed only on those days when the weather is such that honeybees are likely to be foraging. Counting was done over the whole of the zone by assessing the number of bees passing an area of 60cm wide, whilst walking at a normal speed down the side of the zone. The foraging activity of the bees was expressed as the number of bees observed separately within each zone on each assessment. After the adaptation period of the beehive, the foraging activity was evaluated generally twice a day at regular intervals. In addition, the day of treatment foraging activity is assessed around 15 minutes before treatment and around 15 minutes and 1 hour after each treatment except for tunnel n°3 where the item was applied late in the evening out of any foraging activity.

Before the foraging assessments, when possible, were performed within each tunnel the number of bees leaving and entering the hive were recorded over a five minute period by an observer equipped with clicker counters. In order to avoid any mistake, counting of bees entering the hive was done for a 2min 30s period, then counting for bees leaving the hive for a 2min 30s period with another second sequence of 5 minutes.

Duration of flower visits was assessed during same time number of the assessment bees leaving and entering the hive. This was performed by recording the time (seconds) 15 different bees forage over 15 different attractive plants (This was done for 15 bees with a maximum time of 30 seconds in order not to delay the following assessments). The plants chosen for this assessment were chosen without conscious bias from those available within each tunnel.

Behaviour of bees was observed during assessment of bee mortality, foraging activity and control of the colony. Bees were observed for abnormalities like aggressiveness, intensive flying without landing on the crop, moribund or severely affected bees, bee accumulation at the beehive entrance, trembling, bees no longer producing pollen balls, etc.

Assessments on the control of the colony were made on the day of their installation within the tunnels, July 1st and at the end of the exposure phase, July 14th, the day before returning beehives to the beekeeper.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Cumulative number of dead bees before as well as after the applications in the control, the two test item groups and the reference item group, respectively
- Number of foraging bees/m^2 before as well as after the applications in the control, the two test item groups and the reference item group respectively
- Behaviour of the bees during assessment in the control, the two test item groups and the reference item group, respectively
- Bees leaving and entering the hives in the control, the two test item groups and the reference item group, respectively
- Duration of flower visits in the control, the two test item groups and the reference item group respectively
- Control of the colony with the following criteria examined: weight of the empty frame introduced into the centre of the hive, the percentage frame surface area containing honey for both sides of each frame, the percentage frame surface area containing pollen for both sides of each frame, the percentage frame surface area containing eggs for both sides of each frame, for both sides of each frame the percentage surface area of brood (young and old larvae) in each frame and % of capped and uncapped alveolus as well as the health of the queen.

Dates of Work: 01st July to 14th July 2005

Findings:

Honey Bee Mortality:

A summary of the daily mortality (cumulated dead bees) is shown in following table.

Table 3: Cumulated dead bees during the study period (only males and worker-bees considered)

Treatments		Dates					
		05.07.05 3DBT	06.07.05 2DBT	07.07.05 0DBT	08.07.05 1DAT	10.07.05* 1+2DAT	11.05.05 5DAT
Tunnel No. 1 Water	Males	9	15	19	26	35	37
	Workers	367	514	640	770	957	1154
	Total	**376**	**529**	**659**	**796**	**992**	**1191**
Tunnel No. 2 Deltamenthrin EW 15 @ 7.5 g a.s./ha Treatment during foraging activity	Males	11	12	15	22	25	55
	Workers	717	902	1049	1464	1839	2133
	Total	**728**	**914**	**1064**	**1486**	**1864**	**2188**
Tunnel No. 3 Deltamenthrin EW 15 @ 7.5 g a.s./ha evening Treatment out of foraging activity	Males	5	6	7	8	10	13
	Workers	517	656	773	995	1347	1575
	Total	**522**	**662**	**780**	**1003**	**1357**	**1588**
Tunnel No. 6 Dimethoate @ 400 g a.s./ha	Males	10	11	15	20	27	36
	Workers	1297	1555	1788	4501	6514	7467
	Total	**1307**	**1566**	**1803**	**4521**	**6541**	**7503**

DBT = days before treatment
DAT = days after treatment;
* Counting of two days 09. and 10.07.05

During the adaptation phase bee mortality was medium. The day before application, the different level of mortality were medium to low and homogeneous, the toxic standard tunnel having the highest mortality but in a normal range. In the water (control) tunnel, mortality was stable after treatment and quite low. In the dimethoate tunnel, mortality was high after treatment with 2718 dead bees and stayed relatively high.

In this context, Deltamethrin EW 15 applied during foraging activity showed a relatively slight toxic effect the day of application. Nevertheless, this toxic effect was, by far, lower than the toxic standard toxicity.

When applied out of any foraging activity period, Deltamethrin EW 15 showed no effect the day following the application and a very limited effect two days later. The I_{tox} values were quite low in both cases (Deltamethrin EW 15, 24 h after treatment: 2.7; 96 h after treatment: 1.7; Deltamethrin EW 15-evening: 24 h after treatment: 1.9; 96 h after treatment: 2.0). The level of mortality stayed at a very acceptable level taking into account the high level of mortality in the toxic standard tunnel.

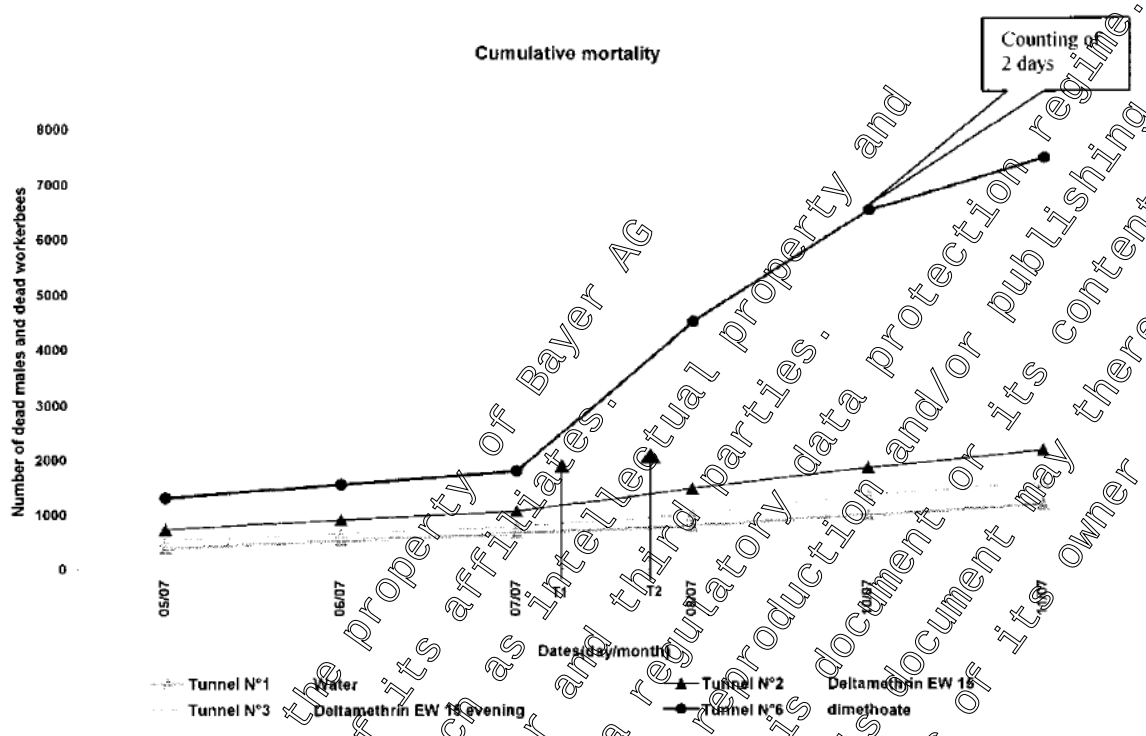


Figure 1: Cumulative mortality of bees (T1 = treatment during foraging activity, T2 = treatment without foraging activity): tunnel No. 1 – water control, tunnel No. 2- Deltamenthrin EW 15 at 7.5 g a.s./ha (treatment during foraging activity), tunnel No. 3- Deltamenthrin EW 15 at 7.5 g a.s./ha evening (treatment out of foraging activity) and tunnel No. 6- reference item

Foraging activity

Deltamethrin EW 15 had no or a very limited impact on foraging activity except in the hours or the day following the application when applied during foraging activity. This impact was somewhat longer, one day more, when Deltamethrin EW 15 was applied out of any foraging activity.

In the same time, the foraging activity in the dimethoate tunnel was almost nil that had to be considered as normal for a toxic standard.

Table 4: Number of bees foraging in the treated zones (Tl, T2, T3 and T4): tunnel No. 1 – water control, tunnel No. 2- Deltamenthrin EW 15 at 7.5 g a.s./ha (treatment during foraging activity), tunnel No. 3- Deltamenthrin EW 15 at 7.5 g a.s./ha evening (treatment out of foraging activity) and tunnel No. 6- reference item

Assessment date (Day/month/hour)	Number of bees/m² (means)			
	Tunnel N°1 Water	Tunnel N°2 DELTAMETHRIN EW 15	Tunnel N°3 DELTAMETHRIN EW 15 evening	Tunnel N°6 Dimethoate
05/07 - 11h43-12h24	9,08	4,89	6,06	5,70
05/07 - 15h51-16h33	9,44	8,27	8,81	11,20
06/07 - 15h00-15h41	5,75	7,38	6,27	6,88
06/07 - 19h34-20h27	2,95	4,44	3,13	4,55
07/07 - 11h13-11h49	4,08	3,78	3,42	7,81
07/07 - 14h15-14h45	13,06	9,05		10,97
07/07 - 14h52-15h13	5,72	0,42	-	5,39
07/07 - 15h37-15h56	8,81	1,08	-	0,45
07/07 - 17h26-18h00	2,52	0,78	1,61	0,14
08/07 - 14h15-15h05	15,59	6,81	3,52	1,69
08/07 - 17h41-18h14	1,31	7,11	2,17	0,05
09/07 - 16h10-16h59	7,16	7,36	3,95	1,8
10/07 - 14h33-15h50	16,77	15,34	10,17	0,63
10/07 - 17h31-18h36	18,13	9,39	8,83	0,75
11/07 - 11h00-11h42	13,89	14,84	16,17	0,56
11/07 - 16h00-16h46	21,80	18,11	18,83	3,22
12/07 - 11h57-12h16	10,56	6,77	9,95	0,06
12/07 - 14h54-15h22	23,87	21,84	24,31	0,88

Treatment

Bees leaving and entering the beehives

Compared to the water (control) tunnel, no negative impact on bee entrance or leaving numbers was seen with Deltamethrin EW 15 except in the hours or the day following the application when applied during foraging activity. This impact was also somewhat longer when Deltamethrin EW 15 was applied out of any foraging activity. On the contrary, in the toxic standard tunnel, a negative impact was observed during all the exposure phase showing that there was more or less no or a very limited activity within the tunnel.

Table 5: Bees entering the beehives in the different tunnels: tunnel No. 1 – water control, tunnel No. 2- Deltamenthrin EW 15 at 7.5 g a.s./ha (treatment during foraging activity), tunnel No. 3- Deltamenthrin EW 15 at 7.5 g a.s./ha evening (treatment out of foraging activity) and tunnel No. 6- reference item

Assessment date (Day/month/hour)	Number of bees entering the beehives in two times 2.5 minutes			
	Tunnel N°1 Water	Tunnel N°2 DELTAMETHRIN EW 15	Tunnel N°3 DELTAMETHRIN EW 15 evening	Tunnel N°6 dimethoate
05/07 - 11h43-12h24	155,00	160,00	112,00	169,00
05/07 - 15h51-16h33	181,00	134,00	245,00	126,00
06/07 - 15h00-15h41	87,00	110,00	154,00	130,00
07/07 - 11h13-11h49	79,00	54,00	109,00	80,00
07/07 - 14h15-14h45	-	-	-	-
07/07 - 14h52-15h13	-	-	-	-
07/07 - 15h37-15h56	-	-	-	-
07/07 - 17h26-18h00	213,00	55,00	47,00	46,00
08/07 - 14h15-15h05	241,00	272,00	179,00	115,00
08/07 - 17h41-18h14	206,00	74,00	55,00	84,00
10/07 - 14h33-15h50	303,00	226,00	167,00	106,00
10/07 - 17h31-18h36	177,00	257,00	178,00	136,00
11/07 - 11h00-11h42	199,00	208,00	181,00	44,00
11/07 - 16h00-16h46	248,00	264,00	282,00	186,00

Table 6: Bees leaving the beehives in the different tunnels: tunnel No. 1 – water control, tunnel No. 2- Deltamenthrin EW 15 at 7.5 g a.s./ha (treatment during foraging activity), tunnel No. 3- Deltamenthrin EW 15 at 7.5 g a.s./ha evening (treatment out of foraging activity) and tunnel No. 6- reference item

Treatment

Assessment date (Day/month/hour)	Number of bees leaving the beehives in two times 2.5 minutes			
	Tunnel N°1 Water	Tunnel N°2 DELTAMETHRIN EW 15	Tunnel N°3 DELTAMETHRIN EW 15 evening	Tunnel N°6 dimethoate
05/07 - 11h43-12h24	83,00	59,00	41,00	109,00
05/07 - 15h51-16h33	99,00	63,00	33,00	68,00
06/07 - 15h00-15h41	87,00	192,00	109,00	28,00
07/07 - 11h13-11h49	25,00	79,00	46,00	122,00
07/07 - 14h15-14h45	-	-	-	-
07/07 - 14h52-15h13	-	-	-	-
07/07 - 15h37-15h56	-	-	-	-
07/07 - 17h26-18h00	13,00	45,00	36,00	28,00
08/07 - 14h15-15h05	278,00	222,00	112,00	34,00
08/07 - 17h41-18h14	29,00	233,00	18,00	50,00
10/07 - 14h33-15h50	187,00	276,00	56,00	46,00
10/07 - 17h31-18h36	133,00	96,00	55,00	40,00
11/07 - 11h00-11h42	74,00	220,00	223,00	16,00
11/07 - 16h00-16h46	139,00	217,00	155,00	118,00

Duration of flower visits

The time (in seconds) that 15 different bees forage over 15 different attractive plants was recorded. The plants chosen for this assessment were chosen without conscious bias from those available within each tunnel.

The results did not show any evident effect of Deltamethrin EW 15 on the duration of flower visits. In the toxic standard tunnel, a negative impact was observed only the day following the application but this had to be counterbalance with no or a very limited foraging activity during all the exposure phase.

Table 7: Mean duration of flower visit (based on 15 bees)

Assessment date (Day/month/hour)	Duration of flower visit in second (means of 15 bees)			
	Tunnel N°1 Water	Tunnel N°2 DELTAMETHRIN EW 15	Tunnel N°3 DELTAMETHRIN EW 15 evening	Tunnel N°5 dimethoate
05/07 - 11h43-12h24	6,80	6,33	9,19	7,33
05/07 - 15h51-16h33	4,20	12,37	7,90	4,28
06/07 - 15h00-15h41	3,71	6,93	8,60	2,75
06/07 - 19h34-20h27	4,26	5,48	5,07	2,21
07/07 - 11h13-11h49	4,49	7,02	5,83	4,53
07/07 - 17h26-18h03	7,29	6,01	7,71	0,00
08/07 - 14h15-15h05	4,43	2,02	2,43	1,59
08/07 - 17h41-18h14	5,27	8,66	6,39	0,00
10/07 - 14h33-15h50	4,30	8,46	5,96	4,91
10/07 - 17h31-18h36	5,19	6,01	7,85	3,62
11/07 - 11h00-11h42	4,19	3,73	3,91	3,47
11/07 - 16h00-16h46	6,52	8,14	5,61	3,16
12/07 - 11h57-12h19	4,57	6,44	4,96	2,21
12/07 - 14h54-15h22	3,15	8,70	5,23	5,26

Treatment

Behaviour of the bees

Deltamethrin EW 15 showed no effect on bee behaviour except the day of application and the following morning for the application done during foraging activity and the day after for the application done out of any foraging activity. During that time, bees were avoiding the crop, flying on the crop without any landing. In the toxic standard tunnel, no or a very limited activity at all was observed during all the exposure phase. Only dead bees were seen.

Before the hives were returned to the apiary, the examined criteria showed that the treatment effect on the bee colony was nil for Deltamethrin EW 15 whatever the type of application. On the contrary, a strong impact of the treatment was observed on the bee colony belonging to the toxic standard tunnel.

Control of the colony

Reserve reduction (honey and pollen) and brood reduction were nil in the water (control) tunnel. This was the same thing for Deltamethrin EW 15 whatever the type of application. On the contrary, there were limited reserves in the toxic standard tunnel. At the end, the queen was seen in all the tunnels.

Assessments of the control of the colony are listed in the following tables.

Table 8: Control of the colony exposed to Deltamethrin EW 15 treated Phacelia at 7.5 g a.s./ha (during foraging activity)

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	500	500
% frame surface area containing honey	Side a	0	0	15	15	15	15	15	15	10	10	5	10	0	0
	Side b	0	0	15	15	20	15	15	15	10	10	10	10	0	0
% frame surface area containing pollen	Side a	0	0	10	5	10	10	10	5	5	5	0	0	0	0
	Side b	0	0	0	0	10	5	10	5	10	5	0	0	0	0
% frame surface area containing eggs	Side a	NR	NR	20	10	20	15	5	5	10	10	NR	NR	NR	NR
	Side b	NR	NR	15	10	10	10	15	10	10	10	NR	NR	NR	NR
% surface area of brood	Side a	0	0	60	60	60	60	80	60	20	30	0	0	0	0
	Side b	0	0	60	60	50	70	60	60	30	40	0	0	0	0
% capped alveolus	Side a	NR	NR	50	80	20	40	50	70	0	30	NR	NR	NR	NR
	Side b	NR	NR	50	70	60	80	30	50	0	50	NR	NR	NR	NR
% uncapped alveolus	Side a	NR	NR	50	20	80	60	80	30	100	70	NR	NR	NR	NR
	Side b	NR	NR	50	30	40	20	50	50	100	50	NR	NR	NR	NR
		T		T		T		T		T		T		T	

NA = Not available, NR = Not relevant, T = Treatment

Table 9: Control of the colony exposed to Deltamethrin EW 15 treated Phacelia at 7.5 g a.s./ha (in the evening out of foraging activity)

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	500	500
% frame surface area containing honey	Side a	0	0	10	10	15	15	15	15	5	10	5	5	0	0
	Side b	0	0	15	10	15	10	15	10	10	10	0	10	0	0
% frame surface area containing pollen	Side a	0	0	10	5	5	5	10	5	5	5	0	10	0	0
	Side b	0	0	5	5	10	0	10	5	0	5	0	0	0	0
% frame surface area containing eggs	Side a	NR	NR	5	5	15	5	10	NR	5	10	NR	30	NR	NR
	Side b	NR	NR	10	10	10	10	10	2	NR	50	NR	NR	NR	NR
% surface area of brood	Side a	0	0	60	60	60	60	50	50	20	30	0	10	0	0
	Side b	0	0	60	60	60	60	70	50	0	20	0	0	0	0
% capped alveolus	Side a	NR	NR	20	5	50	60	80	100	10	50	NR	10	NR	NR
	Side b	NR	NR	60	50	60	80	50	70	NR	10	NR	NR	NR	NR
% uncapped alveolus	Side a	NR	NR	80	95	50	40	20	0	90	50	NR	90	NR	NR
	Side b	NR	NR	40	50	40	20	50	30	NR	90	NR	NR	NR	NR
		T		T		T		T		T		T		T	

NA = Not available, NR = Not relevant, T = Treatment

Table 10: Control of the colony exposed to the water treated Phacelia

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07	01.07	14.07
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	400	700
% frame surface area containing honey	Side a	0	0	15	15	15	15	15	20	10	15	10	10	0	0
	Side b	0	0	10	20	15	20	15	20	10	15	15	10		0
% frame surface area containing pollen	Side a	0	0	10	5	10	5	10	5	5	4	3	5	0	
	Side b	0	0	5	5	10	10	10	10	10	5	0	5	0	0
% frame surface area containing eggs	Side a	NR	NR	5	10	30	15	10	20	NR	10	NR	20	NR	NR
	Side b	NR	NR	10	10	10	10	10	30	NR	30	NR	30	NR	NR
% surface area of brood	Side a	0	0	60	70	80	80	80	80	15	40	0	30	0	0
	Side b	0	0	70	60	80	80	80	80	20	60	0	20	0	0
% capped alveolus	Side a	NR	NR	60	80	50	70	70	20	100	80	NR	0	NR	NR
	Side b	NR	NR	70	70	50	70	50	20	100	70	NR	0	NR	NR
% uncapped alveolus	Side a	NR	NR	40	30	50	50	30	80	0	70	NR	100	NR	NR
	Side b	NR	NR	30	30	50	30	50	80	0	80	NR	100	NR	NR
		T		T		T		T		T		T		T	

NA = Not available, NR = Not relevant, **T** = Treatment

Conclusion:

Based on the different results observed in the water tunnel and in the dimethoate tunnels, this study had to be considered as a valid study. Water application was not toxic on bees whereas dimethoate (reference item) was very toxic. In addition, the level of foraging activity was high.

During the adaptation phase bee mortality was medium. The day before application, the different level of mortality were medium to low and homogeneous, the toxic standard tunnel having the highest mortality but in a normal range. In the water (control) tunnel, mortality was stable after treatment and quite low. In the dimethoate tunnel, mortality was high after treatment with 2718 dead bees and stayed relatively high. In this context, Deltamethrin EW 15 applied during foraging activity showed a relatively slight toxic effect the day of application. Nevertheless, this toxic effect was, by far, lower than the toxic standard toxicity. When applied out of any foraging activity period, Deltamethrin EW 15 showed no effect the day following the application and a very limited effect two days later. The Itox values were quite low in both cases, the level of mortality stayed at a very acceptable level taking into account the high level of mortality in the toxic standard tunnel.

Deltamethrin EW 15 had no or a very limited impact on foraging activity except in the hours or the day following the application when applied during foraging activity. This impact was somewhat longer, one day more, when Deltamethrin EW 15 was applied out of any foraging activity. In the same time, the foraging activity in the dimethoate tunnel was almost nil that had to be considered as normal for a toxic standard.

Compared to the water (control) tunnel, no negative impact on bee entrance or leaving numbers was seen with Deltamethrin EW 15 except in the hours or the day following the application when applied during foraging activity. This impact was also somewhat longer when Deltamethrin EW 15 was applied out of any foraging activity. On the contrary, in the toxic standard tunnel, a negative impact was observed during all the exposure phase showing that there was more or less no or a very limited activity within the tunnel.

The results did not show any evident effect of Deltamethrin EW 15 on the duration of flower visits. In

the toxic standard tunnel, a negative impact was observed only the day following the application but this had to be counterbalance with no or a very limited foraging activity during all the exposure phase.

Finally, Deltamethrin EW 15 showed no effect on bee behaviour except the day of application and the following morning for the application done during foraging activity and the day after for the application done out of any foraging activity. During that time, bees were suspicious of the crop, flying on the crop without any landing.

In the toxic standard tunnel, no or a very limited activity at all was observed during all the exposure phase. Only dead bees were seen.

Before return to the apiary, the examined criteria showed that the treatment effect on the bee colony was nil for Deltamethrin EW 15 whatever the type of application. On the contrary, a strong impact of the treatment was observed on the bee colony belonging to the toxic standard tunnel.

Reserve reduction (honey and pollen) and brood reduction were nil in the water (control) tunnel. This was the same thing for Deltamethrin EW 15 whatever the type of application. On the contrary, there were limited reserves in the toxic standard tunnel. At the end, the queen was seen in all the tunnels.

Thus, in conclusion, Deltamethrin EW 15 showed clearly, that this kind of active substance (pyrethroids) has a slight to moderate impact on bees mainly the day of the application when applied directly on bees (during the foraging activity) and less toxicity when applied out of any foraging period. Impact on bee colony was nil.

*****

Report:	**KCP 10.3.1.5.03, [redacted]; 2000**
Title:	Evaluation of impact AE F032640.00 EW01 B106 on honey bees (insectproof tunnels on phacelia crop)
Document No:	M-198218-01-1 (Rep. No.: 2000-23-3)
Guidelines:	CEB 129
GLP:	yes

~~Material and Methods:~~

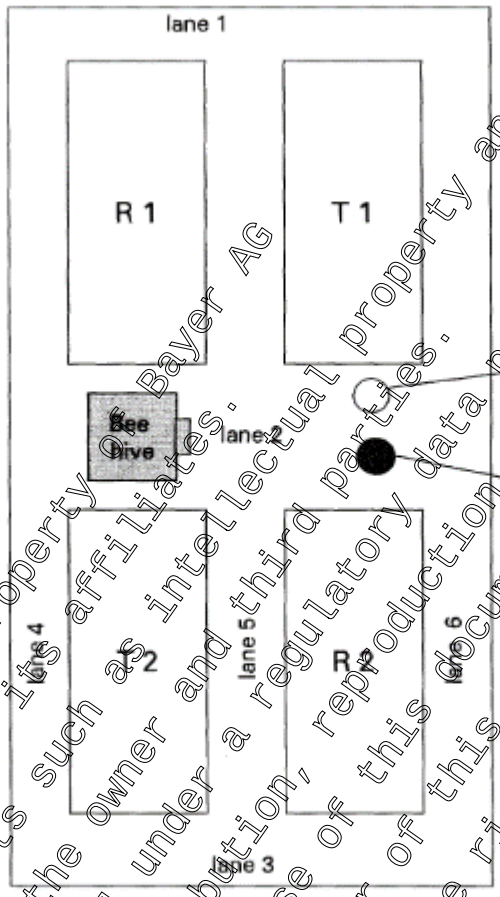
~~Honey bee colonies (ca 17,000 bees per hive, colonies of comparable development, brood, and food store status, queens of homogeneous maternal origin) were confined in tunnels on *Phacelia* fields with additional pollen sources provided. Five days after introduction of the bees into the tunnels, application was performed. The test substance was applied at rates of 7.5 g a.i./ha, the toxic standard was Zolone Flo (500 g/L phosalone). Furthermore, a water-treated control was set up. Treatment was carried out during flight activity of the bees. Endpoints observed were foraging activity, behavior, mortality, and colony development.~~

~~Findings:~~

~~Behavior of the bees was only slightly affected by the test item as well as by the standard. Foraging activity was influenced by the test substance only for a short time. Mortality was not increased significantly by the test item; there was a slight and short-term increase of mortality after application, but overall mortality was comparable between treatment and control. The toxic standard, however, led to a longer-lasting increase of mortality. Colony development was not affected by the treatment.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EW 15 G (AEF032640 00 EW01 B106) content of a.s.: deltamethrin: 1.51 % w/w (15.0 g a.s./L nominal), density: 1.023 g/ml
Batch number:	TA161/99PM
Reference item:	Zolone Flo (active ingredient: phosalone, 500 g a.s./L nominal, analysed content: 499 g a.s./L)
Test organism:	Honey bees (*Apis mellifera*) Young honey bee colonies with queens from the local black breed which were one year old. The queens had a common genetic identity as they were sisters (or half-sisters) coming from a single strain. The colonies live in hives of the DADANT 10 frames model. Populations spread over 7 to 8 frames (of which approximately 2 to 4 frames of brood) have been estimated at around 16,000 to 18,000 bees per hive.
Source:	Bee colonies came from the same apiary containing 1,200 hives allowing easy selection of swarms.
Crop:	*Phacelia tanacetifolia* variety: TITAN (bee attractive crop) at flowering stage.
Test location:	[redacted] on a field from [redacted] France
Test unit:	Each tunnel had a half-moon support made from galvanised steel. The surface per unit was 140 m² (7 m x 20 m) and their roof height approximately 3 metres. A polyethylene mesh net (1.2 mm x 1.2 mm) covered the supports. Both ends were made up of the same material. Access was possible through a zip opening. Inside the tunnels, the *Phacelia* crop was split into four plots. Each had a surface of 16 m² (2 m x 8 m). Two plots were considered as sheltered areas (R1 and R2; not treated with test item), the other two (T1 and T2) as treated areas. A beehive, a watering place and feeders with pollen were placed in each of the tunnels and supplied daily. Exact interior design of the tunnel is shown in the figure below:



R1/ R2: sheltered area
T1/ T2: treated area

Application rates:	Control (C): water Treatment rate (T): 7.5 g a.s./ ha during foraging activity (11:00 to 11:45 a.m.) Reference rate (R) 1.2 L/ ha (600 g a.s./ha)
	Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).
	The spray volume was 300-315 L/ha in all treatment groups. The sprayer was calibrated before use.
Data sampling:	Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.
Data analysis:	All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.
Deviations from the study plan:	No deviation mentioned.

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperatures favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table.

Table 1: Weather data

	minimum Temperature (°C)	maximum Temperature (°C)	Rainfall (mm)
11 July 2000	12	18	9
12 July 2000	7	22	3.5
13 July 2000	15	23	0
14 July 2000	14	21	1
15 July 2000	12	21	0
16 July 2000	7	22	0
17 July 2000	8	23	0
18 July 2000	8	24	0
19 July 2000	9	25	0
20 July 2000	11	31	0
21 July 2000	14	29	0
22 July 2000	16	28	0
23 July 2000	17	28	0
24 July 2000	17	26	0
25 July 2000	12	21	0

N.B. : Temperatures are given for the day mentioned, whereas rainfall recorded every morning shows precipitation over the previous 24 hours.

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 12: *Phacelia* crop data

Date	Operation	Caracteristics
May	Soil preparation	Herbicide application and harrowing, seedbed preparation and weed destruction
15/05/00	plot sawing - rolling	Specie *Phacelia tanacetifolia* variety : TITAN Reference : H 6 33 / 373 of 1998 OECD system, EC norms, ICIA method Model 3 - 580.923.584 Dose seed 6,5 kg per ha
22/09/99	Destruction	Crushing the crop on experimental plots

The effects of Deltamethrin EW 15 G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the guidance document C.E.B. method no. 129.

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EW 15 G on the honeybee, *Apis mellifera* under forced exposure conditions.

This study included three exposure groups with one replicate (tunnel) each: one water treated control group (tunnel 2), one test-item group (tunnel 4) and one reference item group (tunnel 3). In all exposure groups, the crop was sprayed 7 days after set-up of the hives in the tunnels at flowering stage of the

crop, during which time honeybees were actively foraging on the crop under confined conditions. The honeybees remained 7 days in the tunnels following application.

The hives were introduced into the tunnels five days prior to product application, in order to await a mortality decrease and stabilisation. The colonies were comparable to each other during the first visit at the beginning of the test period, and mortality was homogeneous the first day of the study.

Mortality in each tunnel was recorded on a daily basis for all areas covered with plastic film, from days before treatment (7DBT) to 7 days after treatment (7DAT). Moreover, the day on which product application was carried out (0DBT) additional counts were done at the end of the day (0DAT) in order to establish possible brutal intoxication of foraging bees. The total mortality rate recorded in a tunnel for a given day results from adding up mortality rates observed in each of the six plastic rows in the tunnel.

Foraging was observed from 2DBT to 2DAT in all the sheltered (R1 and R2) and treated areas (T1 and T2). It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts were shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall...etc.) This parameter was also taken into account for an additional count on the day of treatment, during the hour following product application.

Two colony assessments were carried out in the beginning and at the end of experimentation (7DBT and 7DAT), allowing to evaluate colony development taking into account parameters like the adult bee population, the quantity and quality of the brood (different stages observed), amount of reserves and potential construction of new frames on offered wax sheets. These visits were carried out in the tunnels at dates which were as close as possible to the first and last day of confinement. However, for practical or climatic conditions, they necessarily took place within 48 hours before or after introduction of the hives in the tunnels on the one hand, and when the hives were taken out on the other hand.

Assessments of bee behaviour were carried out when products were applied and during the thirty minutes following product application. In general, this observation phase continued all over the day, between counts. Bees were especially observed for reactions and behaviour like intense flying, bee clusters on the net or at the entrance of the hive, aggressiveness, beginning of an intoxication etc. in each of the tunnels.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item group and the reference item group, respectively
- Number of foraging bees/m^2 per day on all the areas (T1, T2 and R1, R2) before as well as after the applications in the control, the test item group and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the test item groups and the reference item group, respectively
- Colony Assessment in the beginning and at the end of experimentation

Dates of Work: 12th July to 25th July 2000

Findings:

Honey bee mortality

A summary of the daily mortality and total mortality results are shown in the following table.

Table 3: Daily mortality data

Treatment	7DBT – 12th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	199	28	57	9	4	135	432
Water control	35	14	43	12	4	77	185
Zolone Flo	290	52	135	41	4	242	764
	6DBT – 13th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	214	59	171	46	16	150	656
Water control	62	62	124	48	15	262	573
Zolone Flo	579	82	300	132	31	407	1531
	5DBT - 14th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	85	19	192	21	20	86	423
Water control	51	22	168	29	19	93	382
Zolone Flo	157	38	218	55	28	187	673
	4DBT - 15th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	205	14	56	17	11	106	409
Water control	149	16	50	31	7	105	358
Zolone Flo	198	15	97	41	15	148	484
	3DBT - 16th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	178	15	22	19	6	41	281
Water control	187	15	31	42	12	127	414
Zolone Flo	216	16	28	18	4	105	387
	2DBT – 17th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	124	2	44	29	1	50	250
Water control	71	10	71	42	7	53	254
Zolone Flo	115	18	88	61	8	54	344
	1DBT - 18th July						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	193	11	15	16	7	31	273
Water control	211	20	24	63	6	48	372
Zolone Flo	203	24	21	64	7	56	375
	0DBT - 19th July morning						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EW 15 (7.5 g a.s./ ha)	164	8	16	24	8	30	250

Water control	180	12	14	39	4	49	**298**
Zolone Flo	316	14	18	69	3	58	**478**
	0DAT – 19th July afternoon						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	321	18	126	80	19	103	**661**
Water control	85	9	44	21	7	26	**191**
Zolone Flo	294	13	40	39	10	96	**492**
20 July	**1DAT – 20th July**						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	147	7	67	34	4	55	**314**
Water control	51	4	15	16	5	8	**99**
Zolone Flo	137	23	49	45	5	36	**295**
21 July	**2DAT – 21st July**						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	152	7	40	10	3	32	**253**
Water control	204	23	47	41	2	59	**376**
Zolone Flo	524	24	78	85	6	89	**806**
	3DAT – 22nd July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	212	5	40	19	55	22	303
Water control	214	16	62	38	10	34	364
Zolone Flo	451	23	52	67	55	73	675
	4DAT – 23rd July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	245	12	34	36	2	27	356
Water control	215	17	48	47	73	49	380
Zolone Flo	454	7	42	75	2	67	648
	5DAT – 24th July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	41	19	52	32	8	54	206
Water control	52	46	62	50	13	46	269
Zolone Flo	74	35	95	94	36	85	419
	6DAT – 25th July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	27	31	142	27	9	96	322
Water control	9	54	309	40	16	134	562
Zolone Flo	75	61	405	114	7	137	799
	6DAT – 26th July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EW 15 (7.5 g a.s./ ha)	47	41	110	26	22	52	298
Water control	22	69	129	36	12	68	336
Zolone Flo	58	76	135	84	28	80	461

DBT: days before treatment
DAT: days after treatment

A figure of the total mortality is displayed in the figure below.

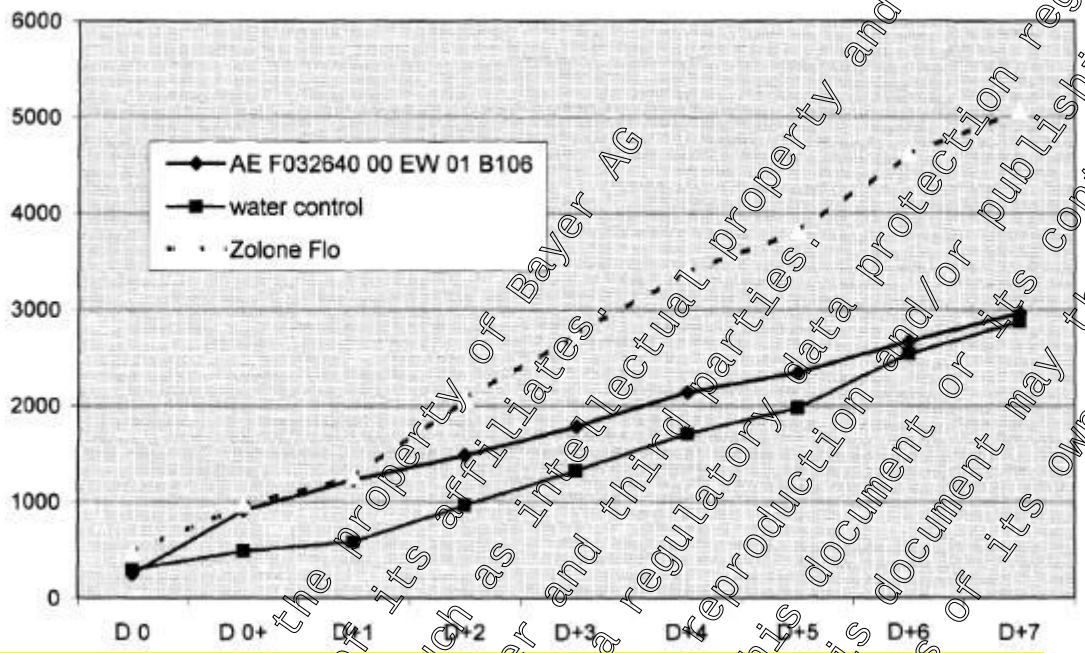


Figure 1: Total mortalities during study period for the reference group, Deltamethrin EW 15 G (AEF032640 00 EW01 B106) at 7.5 g a.s./ha and for the water control group
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+7: 1 to 7 days after treatment

After a week of confinement, mortality rates were comparable for the three tunnels.

The day after the treatment, mortality trends showed quite some differences. Deltamethrin EW 15 and the reference item (Zolone Flo) showed an increasing mortality whereas the water control tunnel showed a regular evolution. In this control tunnel (treated with water) the colony was not disturbed by the treatment. Mortality rates recorded varied very few along the week.

At 1DAT the difference between Deltamethrin EW 15 and the reference item was linked to the duration effect. Deltamethrin EW 15 showed a peak at 1DAT but this phenomenon was very brief. From 2DAT the mortality rates recorded in this tunnel literally dropped to a level which was comparable to the one before treatment and remained stable till the end of the experimental phase.

On the other hand, the standard tunnel treated with the reference item showed an increasing mortality for several days. The daily mortality trend in the tunnel treated with Deltamethrin EW 15 was comparable to that of the control tunnel. There was, however, some increase in mortality rates following product application observed only at 1DAT. This increase in mortality rates had only a limited extent. Until the end of the trial this parameter regularly remained low observed in the other tunnels.

The graph on total mortality rates confirmed these mortality trends. In the tunnel where the test item Deltamethrin EW 15 was used, the curve increased during the first half day following product application. On the contrary, afterwards, progression was quite regular from 1DAT to 7DAT, comparable to the standard tunnel. Most of the dead bees in the final global count were due to the mortality peak on 1DAT.

The effects caused by the reference item Zolone Flo were more complex: immediately after product application, a high mortality rate was observed. Thereafter, daily mortality rates remained high until 7DAT, yielding a total mortality rate which was clearly higher than that of the other tunnel in the end.

Distribution of dead bees within the tunnels did not give any additional information. This distribution was mainly influenced by wind and maybe by the sunlight guiding the bees, because most of the data were recorded in the rows at both ends of the tunnels (A1 or A3).

Foraging activity

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following figure.

Table 4: Foraging data: Deltamethrin EW 15 at 7.5 g a.s./ha

tunnel 4		number of bees per zone								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
17 juil 00	10h00	33	71	42	46	37	17	26	52	96	66	6,0	4,1
D-2	11h00	204	171	181	206	147	194	229	198	381	384	23,8	24,0
	14h00	249	258	213	222	152	218	221	251	471	471	29,4	29,3
											290		18,1
18 juil 00	9h30	92	75	60	82	62	85	106	94	155	174	9,7	10,8
D-1	10h30	186	197	162	172	212	148	209	219	361	394	22,6	24,6
	12h00	249	216	223	224	214	160	197	217	456	394	28,5	24,6
											321		20,0
19 juil 00	9h45	90	108	97	74	88	54	76	106	190	162	11,8	10,1
D 0	10h45	195	182	235	178	201	158	222	187	396	384	24,7	24,0
											273		17,1
	12h00	166	201	158	178	124	51	64	72	352	156	22,0	9,7
	14h30	162	196	145	201	175	142	147	122	352	293	22,0	18,3
	15h30	238	183	247	196	241	190	235	170	432	418	27,0	26,1
											289		18,1
20 juil 00	9h30	52	103	64	84	63	58	49	86	152	127	9,5	7,9
D+1	10h30	99	175	182	150	170	86	107	148	303	256	18,9	16,0
	14h30	247	208	267	264	231	188	245	176	483	420	30,2	26,3
											268		16,7
21 juil 00	10h00	204	196	201	176	176	144	163	170	386	327	24,1	20,4
D+2	11h00	214	198	213	144	162	150	195	140	385	324	24,0	20,2
	14h30	257	226	287	238	248	180	283	224	501	468	31,3	29,2
											373		23,3

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m²: number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation: R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².

Table 5: Foraging data: Water Control

tunnel 4		number of bees per zone								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
17 juil 00	10h00	33	71	42	46	37	17	26	52	96	66	6,0	4,1
D-2	11h00	204	171	181	206	147	194	229	198	381	384	23,8	24,0
	14h00	249	258	213	222	152	218	221	251	471	471	29,4	26,3
											290		18,1
18 juil 00	9h30	92	75	60	82	62	85	106	94	155	174	9,7	10,8
D-1	10h30	186	197	167	172	212	148	209	219	361	394	22,6	24,6
	12h00	249	216	223	224	214	160	197	217	456	394	28,5	24,6
											321		20,0
19 juil 00	9h45	90	118	97	74	88	54	76	106	190	162	11,8	10,1
D 0	10h45	195	182	235	179	201	158	222	187	296	384	24,7	24,0
											273		17,1
	12h00	166	201	158	178	124	51	64	72	352	156	22,0	9,7
	14h30	162	196	145	201	175	142	147	122	352	293	22,0	18,3
	15h30	238	183	247	196	241	190	235	170	432	418	27,0	26,1
											289		18,1
20 juil 00	9h30	52	103	64	84	53	56	49	86	152	127	9,5	7,9
D+1	10h30	99	175	102	150	170	86	107	148	303	256	18,9	16,0
	14h30	247	208	267	244	231	188	245	175	483	420	30,2	26,3
											268		16,7
21 juil 00	10h00	204	196	201	170	176	144	163	170	386	327	24,1	20,4
D+2	11h00	214	198	213	161	162	150	195	140	385	324	24,0	20,2
	14h30	257	220	287	238	248	180	283	224	501	468	31,3	29,2
											373		23,3
20 juil 00	9h30	178	85	100	115	83	132	108	82	239	243	14,9	15,2
D+1	10h30	206	98	152	115	160	125	215	74	286	287	17,8	17,9
	14h30	328	195	243	185	220	176	215	120	426	366	26,6	22,8
											299		18,7
21 juil 00	10h00	93	140	85	90	124	98	79	95	204	198	12,8	12,4
D+2	11h00	233	200	211	137	221	172	160	212	391	383	24,4	23,9
	14h30	273	248	216	252	255	200	227	250	495	466	30,9	29,1
											349		21,8

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m^2 : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation: R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m^2.

Table 6: Foraging data: Reference item Zolone Flo

Tunnel 3		number of bees per zone								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
17 juil 00	10h00	32	34	19	15	15	19	13	31	50	39	3,1	2,4
D-2	11h00	162	168	135	156	116	114	155	189	311	287	19,4	17,9
	14h00	233	230	192	226	186	201	236	256	441	440	27,5	27,5
											255		15,9
18 juil 00	9h30	111	151	124	91	145	109	96	153	239	252	14,9	15,7
D-1	10h30	186	201	135	219	130	176	172	223	371	351	23,2	21,9
	12h00	248	278	198	263	172	174	220	257	494	412	30,8	25,7
tunnel 4		number of bees per zone								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
17 juil 00	10h00	33	71	42	46	37	17	25	52	96	66	6,0	4,1
D-2	11h00	204	171	181	206	140	194	229	198	381	384	23,8	24,0
	14h00	249	258	213	222	152	218	221	231	471	421	29,4	26,3
											290		18,1
18 juil 00	9h30	92	75	60	82	62	85	106	94	155	174	9,7	10,8
D-1	10h30	186	197	167	172	212	148	209	219	361	394	22,6	24,6
	12h00	249	216	223	224	214	160	197	217	456	394	28,5	24,6
											321		20,0
19 juil 00	9h45	90	118	97	74	88	54	76	106	190	162	11,9	10,1
D 0	10h45	195	182	235	179	201	158	222	187	396	384	24,7	24,0
											273		17,1
	12h00	166	201	158	178	124	51	64	72	352	156	22,0	9,7
	14h30	182	196	145	201	175	142	147	122	352	293	22,0	18,3
	15h30	238	183	247	196	241	190	235	170	432	418	27,0	26,1
											289		18,1
20 juil 00	9h30	52	103	64	84	63	56	49	86	152	127	9,5	7,9
D+1	10h30	99	175	182	150	170	86	107	148	303	256	18,9	16,0
	14h30	247	208	267	244	231	188	245	176	483	420	30,2	26,3
											268		16,7
21 juil 00	10h00	204	190	201	170	176	144	163	170	386	327	24,1	20,4
D+2	11h00	214	198	213	140	162	150	195	140	385	324	24,0	20,2
	14h30	257	220	287	238	248	180	293	224	501	468	31,3	29,2
											373		23,3

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation: R = [(R1t + R2t) / 2] / 16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².

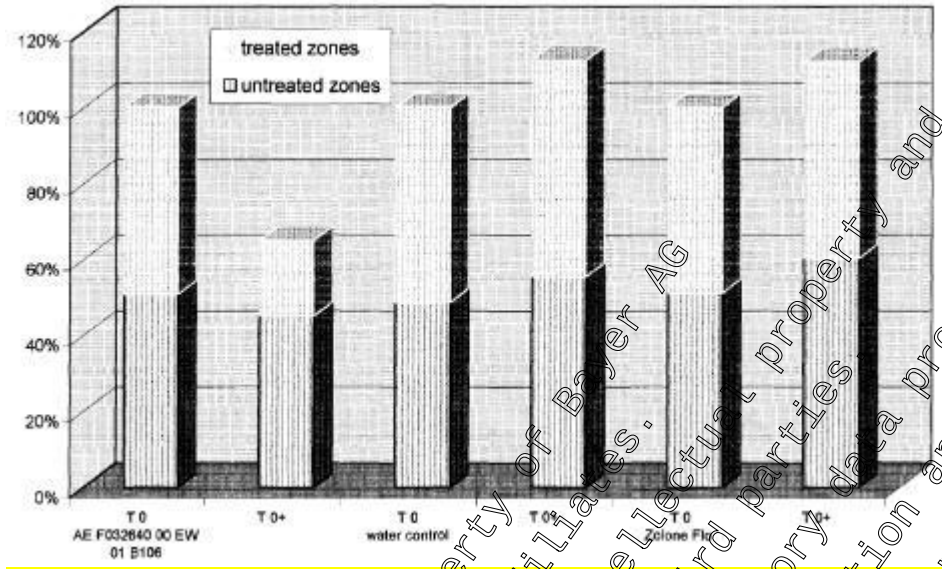


Figure 2: Breakdown of foraging on treatment day for the reference group (Zolone Flo), Deltamethrin EW 15 G (AEF032640 00 EW01 B106) at 7.5 g a.s./ha and for the water control group in treated and untreated zones
T0: before treatment
T0+: after treatment

At the beginning of the experimental phase, following introduction of the bee hives in the tunnels, bees foraged floral buds quite actively. Mean daily thresholds of 15 to 20 bees per m² were reached during the five days before product application.

During the three counts that followed product application, mean foraging trends were different between tunnels. In the tunnel where the test item Deltamethrin EW 15 was applied the activity decrease but did not stop and the average level in the afternoon was even higher than 18 bees per m².

The colony in the reference tunnel seemed indifferent to reference item application, and foraging increased during the day over pre-treatment phase levels. On the same way in the control tunnel, where water spraying did not disturb the foragers' activity, it slightly increased during the day. On the following day (at 1DAT) foragers' activity was slowed down after product application in these two tunnels where standard product and control were used, reaching the pre-application activity level.

In the Deltamethrin EW 15 tunnel this activity did not move and stay at the same level the day before. In the three tunnels, foraging thresholds remained quite high two days after product application (2DAT), at comparable levels to those obtained over several days, always about 15 and 20 bees per m² on average, with even higher activity peaks during the day in the most favourable conditions.

Shortly after product application (0|2AT, during the thirty minutes following product application), a minor repulsive effect was observed in the tunnel. The decrease in foraging activity affected treated areas only, whereas sheltered (untreated) areas were visited by the same amount of foragers. This confirms the impact of Deltamethrin EW 15 on foraging activity on average on the treatment day.

On the contrary, the two other tunnels showed increased activity on both sheltered and treated areas, this explains levels over 100 %. However, it is preferable to deal about relative foraging stability in the three tunnels because differences were not significant.

Colony Assessment

There were few differences concerning the structure of the colonies between the two visits. The state of the reserves and proportions of the brood remained stable for the reference item tunnel (Zolone Flo). Brood from the control and test item (Deltamethrin EW 15) tunnels remained also constant, but their reserves slightly decreased. However, these differences were not of sufficient magnitude to reveal a radical change in the colonies behaviour of those two tunnels.

The slight consumption of reserves for the water tunnel and the Deltamethrin EW 15 tunnel could have been induced by the difference between the need for food of the colonies and the restrictive conditions during the tunnel confinement phase.

Despite a high mortality recorded during the trial, in the Zolone Flo tunnel, populations of the remained stable between the two apiarist visits. The increase of mortality, during the treatment period (1DBT to 1DAT), may have been compensated by the emergence of new bees on the brood frames, which would explained the stability of the bees populations.

Behaviour of the bees

Colony behaviour was comparable between tunnels as foraging was quite regular on Phacelia plots. Colonies in the different tunnels only showed little reaction to treatments, if it were not for flying away when the boom with water passed by.

In the tunnels where the test product was used, a few clinical signs occurred in the hour following product application. These signs were observed in the afternoon but not the next or following days.

Activity at the hive entrance was normal in three tunnels. No bee clusters were observed on the nets nor at the hive entrance and no fleeing events were observed in any of the tunnels.

Intoxication symptoms: Foragers in contact with the test item were the ones that were affected first. In the Deltamethrin EW 15 and in the reference item (Zolone Flo) tunnels, some bees were on the ground after treatment and they had typical intoxication signs.

In these tunnels, intoxicated bees fell on the plastic surface of the rows, walked in a difficult way alternating periods of sluggishness and frenzy. Such a bee rolled over itself and appeared unable to lift off. Its fore legs then its hind legs and abdomen appeared to be paralysed. The bee died in a range from a few minutes to a few hours.

Conclusion:

Overall conditions for conducting this experimental phase of the scheme were favourable to bee activity. Climatic and crop conditions were satisfactory. The different parameters observed agree with the results obtained.

During the treatment phase, the test item Deltamethrin EW 15 and the reference item Zolone Flo showed impact on bees deaths compared to the water control. But fundamental difference was that mortality caused by the reference item still showed effects over several days, when test item Deltamethrin EW 15 effect was restricted to the day after product application.

Experimental conditions of the study were quite strict, including confinement and product application carried out during intense foraging activity, on very attractive plots. Only the use of the reference item gave a high mortality.

During this trial on a blooming phacelia the effects of the test item Deltamethrin EW 15 only showed a temporary increase in mortality yielding comparable total mortality rates to those recorded in the control tunnel.

*****

Report:	**KCP 10.3.1.5/04, [redacted]; 2014**
Title:	Determination of Side-Effects of Deltamethrin EW 15B G on Honey Bee (*Apis mellifera* L.) Brood Under Confined Semi-Field Conditions
Document No:	M-477316-01-1 (Rep. No.: S12-00041)
Guidelines:	OECD guidance document No. 75 (2007); No major deviations
GLP:	yes (certified laboratory)

Material and Methods:

Test item: Name: Deltamethrin EW 15B G; Sample description: TQX09629-00; Batch-ID: 2012-000065; content of a.s. (analysed): deltamethrin: 16.14 g/L, 1.58 % w/w (15.0 g/L nominal).

The effects of Deltamethrin EW 15B G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the OECD guidance document No. 75 (2007), with modifications.

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EW 15B G on the honeybee, *Apis mellifera* L. under forced exposure conditions. The crop used for this semi-field study was *Phacelia tanacetifolia*, the study was conducted in [redacted] Germany.

This study included three exposure groups with three replicates (tunnels) each: one tap-water treated control group (C), one test-item group (T) and one reference item group (R). In all exposure groups, the crop was sprayed 4 days after set-up of the hives in the tunnels at BBCH 65 (full-flowering), during honeybees actively foraging on the crop under confined conditions. The target application rate of the test item Deltamethrin EW 15B G corresponded to 7.5 g a.s./ha, tap water was applied in the control group and Insegar 25 WG was applied at a target rate of 600 g product/ha in the reference item group (corresponding to 150 g fenoxycarb per ha). The spray volume was 400 L/ha in all treatment groups. The colony size at set-up was in the range of 6188 – 9188 bees. The honeybees remained 10 days in the tunnels.

The first colony assessment was performed 3 days before set-up of the colonies in the tunnel tents. Subsequently, six further colony assessments were conducted.

The colonies were assessed once before, twice during and four times after the end of the confined exposure phase. The development of the bee brood was assessed in parallel in individual marked brood cells. One assessment before application (Brood Area Fixing Day = BFD0) and four further assessments took place. Mortality, flight intensity and behaviour assessments were conducted daily, starting 3 days before the test item application and continued for seven days after the test item application under confined conditions. Further mortality assessments (in bee traps only) and behaviour assessments were conducted at the monitoring site, after the end of the confinement period until DAA26.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Total and mean number of dead bees on the linen sheets in tunnel tents and in the dead bee traps before as well as after the application in T, C and R, respectively.
  - Flight intensity (mean number of forager bees/m^2 and treatment group on *P. tanacetifolia* before as well as after the applications in T, C and R, respectively.
  - Behaviour of the bees in the crop and around the hive.
  - Condition of the colonies and development of the bee brood (number of bees (strength) and area of the different brood stages and food storage per colony and assessment date).
  - Development of the bee brood assessed in individual brood cells.

Dates of Work: 15 June 2012 to 18 July 2012

Findings:

Mortality and Flight Intensity of Honey Bees, Semi-Field Test under Confined Exposure Conditions

Test item	Deltamethrin EW 15B G		
Test object	*Apis mellifera*		
Treatment group	Control **(C)**	Deltamethrin EW 15B G **(T)**	Reference item Insegar 25 WG **(R)**
Application rate		1 × 7.5 g Deltamethrin a.s./ha at BBCH 65	1 × 150 g Fenoxycarb/ha at BBCH 65
Mean mortality DAA-3 to 0ba [dead bees/day]	15.1	17.3	15.3
Mean mortality DAA0ba [dead bees/day]	36.3	33.7	32.3
Mean mortality DAA0aa [dead bees/day]	22.0	30.3	19.3
Mean mortality DAA0aa to 7 [dead bees/day]	19.2	29.9*	27.5
Mean mortality DAA0aa to 26 [dead bees/day]	15.6	18.4	72.7*
Sum of dead pupae and larvae DAA0aa to DAA26	16 Pu/2La	15 Pu	4574 Pu
Q_M (DAA0aa)	1.4	1.8	1.3
Q_M (DAA0aa to 7)	1.3	1.7	1.8
Daily mean flight intensity DAA-3 to 0ba [bees/m^2]	7.6	9.3	10.5
Daily mean flight intensity DAA0aa [bees/m^2]	17.3	6.0*	16.8
Daily mean flight intensity DAA1 [bees/m^2]	28.5	24.4	24.7
Daily mean flight intensity DAA0aa to 7 [bees/m^2]	22.6	17.0*	19.5

DAA = Days after application
ba = before application
aa = after application

* = statistically significant compared to the control
Pu = pupae
La = larvae

Results and Observations:

Honey Bee Mortality:
After set-up of the colonies inside the tunnels until the day of the test item application (DAA-3 to 0ba), the mean mortality value was 15.1, 17.3, and 15.3 dead bees/day for the treatment group C, T and R, respectively. There are no statistically significant results between the treatment groups concerning mortality during this period (t-test, method pooled, one-sided, $\alpha = 0.05$).
On the day of the application, immediately before the test item application in T and the concurrent application in C and R, respectively (DAA0ba), the mean mortality value was 36.3, 33.7 and 32.3 dead bees/day for the treatment group C, T and R, respectively.
On the day of application, after the application (DAA0aa), the mean mortality value in the treatment group C, T, and R accounted to 22.0, 30.3 and 19.3 dead bees/day, respectively.
One day after application (DAA1) the mean mortality in T (34.7 dead bees/day) was slightly, but statistically significantly higher than the corresponding value in the control group C (15.0 dead bees/day) (t-test, method pooled, one-sided, $\alpha = 0.05$). Also statistically significantly higher than the corresponding value in the control group C (13.7 dead bees/day) (t-test, method pooled, one-sided, $\alpha = 0.05$) were the mean mortality values of T and R on DAA7 with 35.7 dead bees/day in T and 36.3 in R, respectively.
The mean daily mortality value during the confined exposure period after the application (DAA0aa to DAA7) of the test item treatment was slightly increased with a mean value of 29.8 dead bees/day and statistically significantly higher than the corresponding value in the control group C with 19.2 dead bees/day (t-test, method pooled, one-sided, $\alpha = 0.05$).
During the further monitoring of the colonies outside the tunnels at the remote monitoring location (DAA8 to DAA26), daily mean mortality was in a range from 6.0 to 25.0 dead bees in C, 6.0 to 35.0 dead bees in T and 5.7 to 277.7 dead bees in R. A statistically significantly higher mortality value occurred only on DAA8 during this period of time in the test item treatment group T when compared to the control group C (t-test, method pooled, one-sided, $\alpha = 0.05$). The mortality values of the reference item treatment group were statistically higher throughout DAA10 to DAA15.
During the total time period after the application (DAA0aa to 26), the mean daily mortality was recorded to be 15.6, 18.4 and 72.7 for C, T, and R, respectively. The mean mortality values in the test item treatment group T before (DAA-3 to 0ba) as well as after test item application (DAA0aa to 26) were not statistically significantly different, when compared to the control group C (t-test, method pooled, one-sided, $\alpha = 0.05$).
Additionally, from DAA10 onwards (i.e. the typical point in time to detect the effect of the reference item), dead bees, dead pupae and dead larvae on the bottom board of each hive were counted and added to the dead bees found in the dead bee trap and were included in the calculation. The values from the bottom board of DAA10 are as such the sum of dead bee life stages until that day.
During the daily assessments of mortality (DAA0aa to 26), the sum of dead pupae and larvae, found inside the dead bee traps and on the bottom board (from DAA10 onwards) of the test item group and control group stayed on the same level with 18 and 15 dead pupae and larvae for C and T, respectively. In contrast, 4574 dead pupae were found in the reference item group during this period.
When comparing the mean mortality before application (DAA-3 to DAA0ba) until the day of the test item application, the $Q_{M(DAA0aa)}$ values were 1.5, 1.8 and 1.3 for the treatment groups C, T and R, respectively. The $Q_{M(DAA0aa\ to\ 7)}$ values were 1.3, 1.7 and 1.8 for C, T and R, respectively.

Although the mean mortality in the test item treatment group was compared to control statistically significantly increased one day after test item application (C: 15.0 vs. T: 34.7 dead bees/day) as well as during the confined exposure period after the application (DAA0aa to DAA7, C: 19.2 vs. T: 29.9 dead bees/day), mortality was in absolute terms still on a low, biologically not adverse level. Therefore, it can be concluded that Deltamethrin EW 15B G, even when applied under forced (confined) exposure conditions at a rate corresponding to 7.5 g a.s./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop during application, does not result in biologically adverse effects on mortality.

Honey Bee Flight Intensity:

After set-up of the colonies inside the tunnels until the day of the test item application and the concurrent application in C and R, respectively (DAA-3 to 0ba), the mean daily flight intensity was 7.6, 9.3 and 10.5 bees/m² in C, T and R, respectively. The daily mean flight intensity during this period was in a range from 1.8 to 15.1 forager bees/m² in C, 0.8 to 15.4 in T and 1.9 to 17.7 in R, respectively.

On the day of the test item application after application (DAA0aa), the mean daily flight intensity across 7 assessments within a period of about 6 hours was 17.3, 6.0, and 16.8 honeybees/m², for C, T, and R, respectively, and was statistically significantly reduced in T, when compared to C (t-test, method pooled, one-sided, $\alpha = 0.05$).

One day after the test item application and the concurrent application in C and R, respectively (DAA1), the mean flight intensity was nearly on the same level in all treatment groups with 28.5, 24.4 and 24.7 forager bees/m² in the C, T and R, respectively and there were no statistically significantly differences in the test item treatment group T when compared to the control group C, but statistically significant differences were observed in the reference item group (t-test, method pooled, one-sided, $\alpha = 0.05$).

During the confined exposure period, after test item application and the concurrent application in C and R, respectively (DAA0aa to DAA7), the flight intensity in the test item treatment group T was statistically significantly lower on DAA2 (23.8 forager bees/m²) and DAA3 (10.6 forager bees/m²) when compared to the control group C (DAA2: 28.8 and DAA3: 17.7 forager bees/m²) (t-test, method pooled, one-sided, $\alpha = 0.05$). The mean daily flight intensity during DAA0aa - DAA7 was in a range from 17.3 - 28.8 forager bees/m² in C, 6.0 - 24.4 in T and 13.1 - 27.5 in R, respectively. The corresponding mean daily flight intensity during DAA0aa - DAA7 was 22.6, 17.0 and 19.5 for C, T and R, respectively. The flight activity was statistically significantly lower in T when compared to C (Mann Whitney Exact, one-sided, $\alpha = 0.05$) during this period.

Overall, a slight repellent effect of the test item was indicated in comparison to control by a statistically significantly reduced flight intensity on DAA0 (day of application, after application), during the 2nd and 3rd day after application as well when considering the overall mean value during confinement. The reduction in flight intensity was most apparent immediately after application, but recovered shortly and distinctly thereafter. Overall, flight intensity was in absolute terms on a sufficiently high level, and as such biologically not adversely reduced.

Behaviour of the Bees

No abnormal behaviour was recorded in the control, in the test item and in the reference item treatment group before application (DAA-3 to DAA0ba). The few cramping, inactive / motionless and clustering bees observed during this period are considered as not unusual and did not impair the interpretation of the study. Bees sitting on the linen sheet and motionless wet bees were considered as normal.

In the test item treatment T, shortly after the application on DAA0aa, across 7 observation time points, 22 cramping bees, 27 bees with locomotion / coordination problems and 6 inactive / motionless bees were observed. Furthermore, many bees intensively cleaning themselves were noticed in the test item treatment group at these 7 observation time points. On DAA1, on the first out of three assessments that day, 28 cramping bees, 81 motionless bees and 6 bees with locomotion problems were observed in the three replicates of test item treatment. In the following two assessments, only one motionless bee but mainly normal behaviour of the bees was observed. From DAA2 to DAA26 only occasionally cramping bees, motionless bees, bees with locomotion problems and fighting bees were observed in T.
In the reference item treatment group, shortly after application on DAA0aa across 7 observation time points, 26 intensive cleaning bees, 13 trembling bees, 6 intensive cleaning bees with locomotion problems, 2 motionless bees and one bee trembling with locomotion problems, one bee flying without landing on the crop, one cramping bee and one bee with locomotion problems. One day after application (DAA1), mainly normal behaviour of the bees was observed in the three assessment times per replicate (Ra, Rb, Rc). Only 1 cramping and 3 motionless bees were recorded in this time period.
On the following days during confined exposure (DAA2 to DAA7) mainly normal behaviour was noticed. One cramping bee, one bee with locomotion problems and 1 bee intensively controlled by a guarding bee were observed in this time period in the reference item tents. Additionally, 44 inactive / motionless bees could also be observed, which is probably due to adverse weather conditions.
During the observations from DAA0aa to DAA26, abnormal behaviour was observed in the control group C only on very few occasions, which is considered biologically normal under confined conditions.

Overall, sub-lethal/behavioural effects in the test item treatment group occurred mainly on the day of application, with a clear decline during the following day, and thereafter only occasionally, showing the transient nature of the effect.

Development of Honey Bee Brood in Individual Cells (Digital Image Analysis)
According to the development time of a worker honey bee from egg to adult bee (imago), which normally averages to 21±1 days, it can be expected that young bees will have hatched until the assessment date BFD+21 (i.e. 21 days after the **B**rood Area **F**ixing **D**ay BFD0).
For this particular assessment, about 250 individually marked cells per hive were selected in C, T and R, respectively.
The control (C) and test item treatment colonies Tb and Tc showed a successful development, with rising brood indices throughout the entire assessment period, except for the assessment on BFD+15, where stable values (due to the long development time of the sealed brood) compared to the previous assessment on BFD+11 were observed in all C colonies and in the colonies Tb and Tc. The development of the colony Ta was interrupted in the observed cells between the assessments BFD0 and BFD+6 and the brood indices were 0 from BFD+6 up to BFD+21.
Maybe this development occurred due to unknown mechanical reasons at the assessment on BFD0 or due to individual characteristics of this bee hive (e.g. the marked, small number of eggs had no priority for the colony at this point in time). Considering (i) the equivalent performance of the colonies Tb and Tc when compared to control, (ii) the overall low termination rates in Tb and Tc, (iii) the absence of any difference between T and C in terms of dead larvae and dead pupae as determined in the bee traps and on the bottom boards of each hive in combination with (iv) the overall comparable performance of brood development in all hives in the test item treatment group when compared to the control group - as assessed in a series of six subsequent colony assessments, involving both sides of each comb per hive - a direct, test item related effect appears unlikely.

The brood development of the reference item colonies Ra and Rb was interrupted and the indices decreased to 0 at the first assessment after application (on BFD+6). The colony Rc showed a successful development of the observed cells, with rising brood indices which is rather untypical for colonies treated with the reference item Insegar. Even with this positive development of the marked cells in this particular R-colony, the effect of the reference item treatment became obvious in Rc by the high number of dead pupae (partially with sickle eyes) in the bee traps, by the high number of dead brood in the cells of the individual combs as well as by the high number of dead pupae on the bottom board, in all of the three R-replicates - Ra, Rb and Rc, respectively.
In total, the brood indices were 1.00 in each treatment group at the first BFD assessment and reached mean values of 3.79, 2.68 and 1.53 in C, T and R at the last assessment on BFD+21.
The compensation index is an indicator for the recovery of the brood in those cells which had been emptied before successful hatch. The mean compensation indices of the control were 2.64, 3.06, 3.11 and 4.28 on BFD+6 to BFD+21 and 1.93, 2.36, 2.75 and 3.79 in the test item treatment. In the only replicate in the test item treatment group (Ta) which showed a total loss of the brood in the marked cells at BFD+6, the determined increasing compensation indices of 0.54, 0.57, 1.74 and 2.87 on BFD+6, +11, +15 and +21, indicate recovery from this event and show new egg-laying activity and successful brood development.
In the reference item treatment R, the mean compensation index increased only to 1.11 at BFD+6 and thereafter on BFD+21 to a value of 2.39.
At the last assessment (BFD+21), the mean termination rate was 24.37 % in the control and 46.58 % in T, compared to a mean value of 69.51 % in the reference item treatment R.
Over the entire assessment period, no statistically significant differences of the brood- and compensation indices and the termination rate of the test item treatment group compared to the control was recorded (t-test method pooled, one-sided, $\alpha = 0.05$). Similarly, also no statistically significant difference of the reference item group compared to the control group was noticed, due to the good development of the individual cells of the replicate Rc. However, the high number of dead pupae (partially with sickle eyes) in the bee traps, the high number of dead brood in the cells of the individual combs as well as the high number of dead pupae on the bottom boards show a clear effect of the reference item treatment.

Overall, the quantitative assessments of brood development in individually marked cells revealed that Deltamethrin EW 15B G, when applied under forced (confined) exposure conditions in gauze tunnels at a rate corresponding to 7.5 g a.s./ha during full-flowering of a highly bee-attractive crop (*Phacelia tanacetifolia*) with honey bees actively foraging on the crop during application, does not cause treatment-related adverse effects on honey bee brood development.
This conclusion is in line with the other brood-related parameters as measured during the course of the study (i.e. the absence of any difference between T and C in terms of dead larvae and dead pupae as determined in the bee traps and on the bottom boards of each hive and the overall performance of brood development within all hives in the test item treatment group, when compared to the control group, as assessed in a series of six subsequent colony assessment and by considering all combs per hive).

Summary of the brood and compensation indices and termination rates

Replicate	Brood / Compensation indices at x days after brood area fixing day (BFD)					Termination rate (BFD+21)
	0	+6	+11	+15	+21	[%]
Ca	1.00 / 1.00	3.12 / 3.13	3.92 / 3.93	3.90 / 3.92	4.87 / 4.91	3.20
Cb	1.00 / 1.00	2.81 / 2.81	2.93 / 2.95	2.93 / 2.99	3.67 / 4.00	26.70
Cc	1.00 / 1.00	1.97 / 1.97	2.27 / 2.29	2.27 / 2.42	2.84 / 3.93	43.22
Mean C	**1.00 / 1.00**	**2.63 / 2.64**	**3.04 / 3.06**	**3.03 / 3.11**	**3.79 / 4.28**	**24.37**
STD	0.00 / 0.00	0.60 / 0.60	0.83 / 0.83	0.82 / 0.76	1.02 / 0.55	20.11
Ta	1.00 / 1.00	0.00 / 0.54	0.00 / 0.57	0.00 / 1.74	0.00 / 2.87	100.00
Tb	1.00 / 1.00	2.64 / 2.66	3.31 / 3.33	3.31 / 3.34	4.13 / 4.32	17.65
Tc	1.00 / 1.00	2.58 / 2.58	3.17 / 3.18	3.13 / 3.16	3.91 / 4.27	22.08
Mean T	**1.00 / 1.00**	**1.74 / 1.93**	**2.16 / 2.36**	**2.15 / 2.75**	**2.68 / 3.84**	**46.58**
STD	0.00 / 0.00	1.51 / 1.20	1.87 / 1.55	1.86 / 0.88	2.32 / 0.85	46.32
Ra	1.00 / 1.00	0.00 / 0.45	0.00 / 0.08	0.00 / 0.27	0.00 / 0.74	100.00
Rb	1.00 / 1.00	0.00 / 0.07	0.00 / 0.00	0.00 / 0.38	0.00 / 1.78	100.00
Rc	1.00 / 1.00	2.79 / 2.81	3.67 / 3.68	3.67 / 3.69	4.59 / 4.67	8.54
Mean R	**1.00 / 1.00**	**0.93 / 1.11**	**1.22 / 1.29**	**1.22 / 1.45**	**1.53 / 2.39**	**69.51**
STD	0.00 / 0.00	1.61 / 1.48	2.12 / 2.07	2.12 / 2.04	2.65 / 2.05	52.80

DAA = Days after application
ba = before application
aa = after application
* = statistically significant compared to the control

Strength of the Colonies

The mean number of bees assessed before set-up of the hives (first colony assessment, DAA-7) in the tunnels revealed a mean colony strength with an average of 6875 bees/hive in C [range: 6188 - 7625], 6875 bees/hive in T [range: 6688 – 7125], and 8459 bees/hive in R [range: 8000 – 9188].

At the second colony assessment on DAA-1, the mean colony strength was 6771 bees/hive in C [range: 6063 – 7563], 5875 bees/hive in T [range: 5500 -6375] and 7709 bees/hive in R [range: 6688 to 8813].

At the third colony assessments (DAA5), during the confined exposure period, an increase of the mean number of bees was observed in both C and T (C: 8500 bees/hive, T: 7313 bees/hive), whereas the mean number of bees in R was almost on the same level as on the first assessment with 8605 bees/hive.

At the subsequent colony assessments, after the end of the confined exposure period outside the tunnels, on the remote monitoring location the mean number of bees increased continuously in the C and T colonies up to the sixth colony assessment (DAA20) to 15000 bees in C and 10875 bees in T. On the last colony assessment (DAA26) the number of bees was comparable in both, C and T, with 11708 and 10271 bees, respectively.

On DAA10 and 11 the mean number of bees in the R colonies was nearly on the same level with 11813 and 11771 bees, respectively. On DAA20 the number of bees increased to 12750 and decrease to 8917 bees on DAA26.

The increase of the mean number of bees from the first to the last colony assessment in the C and T was comparable and accounted to +70.3 % and +49.4 %, respectively, whereas the increase in R colonies accounted only to +5.4 %.

The overall development of colony strength of all treatment groups showed fluctuations which are typical for this type of study. The colony strength values at the last assessment in the C and T colonies

were higher compared to the first assessment and showed comparable absolute numbers (C: 11708, T: 10271 bees). Also the relative increase in colony strength until the end of the study was comparable in C and T (C: +70.3 %, T: +49.4 %). As such, no test-item related adverse effects on colony strength were observed.

Development of Brood Area

The mean abundance of brood (sum of cells containing eggs, larvae, and pupae) assessed before set-up (confinement) of the hives (first colony assessment, DAA-7) was 20600 cells/hive for C, 20133 cells/hive for T and 22800 cells/hive for R. At the second colony assessment (DAA-1 / BTD0), before start of exposure, the mean abundance of brood in C, T and R had increased to 23133 cells/hive for C, 20533 cells/hive for T and 24267 cells/hive for R.

At the third colony assessment, during the confined exposure period, on DAA5, the mean abundance of brood in C, T and R was nearly on the same level with 22333, 20933 and 21933 cells/hive in C, T and R, respectively.

On the fourth colony assessment (end of confined exposure, at the monitoring site) on DAA10, the mean abundance of brood in C and T had decreased slightly and in parallel to almost identical 20267 cells/hive for C and 20400 cells/hive for T, whereas the mean abundance of brood decreased noticeably to 15933 cells/hive in the R colonies.

On the fifth colony assessment (DAA14), the mean abundance of brood in C and T had increased in parallel and was again almost on an identical level with 24000 cells/hive for C and 24533 cells/hive for T, whereas in R, with 13533 cells/hive, the mean abundance of brood had further and noticeably decreased. This refers to a clearly detectable effect of the reference item, which is typical for this point in time.

On the following assessments, the mean abundance of brood in C increased to 29200 cells/hive on DAA20 and 29867 cells/hive on DAA26. In the T colonies on DAA20, the mean abundance of brood increased to 28400 cells/hive and on the last colony assessment (DAA26) 26800 cells/hive was recorded.

Brood of all stages (eggs, larvae, capped brood) was present in all colonies at all assessments during the study with the exception of colony Ra, where no larvae were recorded on the third colony assessment at DAA5. The fluctuations of all brood stages were within the range of natural variation and typical for this kind of study.

Overall, honey bee brood development and colony conditions in the test item treatment T were comparable to control during the entire assessment period. No test-item related adverse effect on brood development was observed.

Development of the Food Storage Area

The mean extent of food stores in the colonies (sum of cells containing nectar and pollen) assessed before set-up (confinement) of the hives (first colony assessment, DAA-7) was 18467 cells/hive for C, 14533 cells/hive for T and 11467 cells/hive for R. At the second colony assessment (DAA-1, before start of exposure), the mean extent of food stores decrease slightly in C and increased in T and R (C: 18200 cells/hive, T: 17333 cells/hive, R: 15733 cells/hive). At the third colony assessment, during the confined exposure period, on DAA5, the mean extent of food stores in the colonies C, T and R had decreased to almost identical levels, i.e. 15867 cells/hive in C, 15333 cells/hive in T and 15533 cells/hive in R).

At the subsequent colony assessments on the remote monitoring location, after the confined exposure period, the mean extent of food stores in C, T and R decreased from DAA10 to DAA26 to finally 8867 cells/hive in C, 6267 cells/hive in T and 8333 cells/hive in R.

The observed de- and increase in food stores in both, treatment and control, during confinement and thereafter can be considered as typical for this type of study. The colonies were well provided during the course of the study. No test-item related adverse effects on the development of the food storage area were observed.

Conclusion:
Deltamethrin EW 15B G was applied at a rate corresponding to 7.5 g a.s./ha during full-flowering to the highly bee-attractive crop *Phacelia tanacetifolia* with honey bees actively foraging on the crop during application. The effects on bee hives under confined exposure conditions considering mortality, flight intensity, behaviour, colony strength and brood development were evaluated.
The tested Deltamethrin EW 15B G application rate has not caused adverse effects on the survival of marked eggs (brood termination rate), on brood development from eggs into adult bees (brood index) as well as on the brood compensation ability (brood compensation index).
Overall, the employed application scenario did not result in test item related adverse effects on brood development, on colony development and on overall colony vitality under forced exposure conditions.
A repellent effect of the test item was indicated by reduced flight intensity on the day of the test item application as well as on two further days during the confined exposure period, but the observed repellent effect was not biologically adverse.
The mortality values in the test item treatment group during the post-application phase were slightly increased on three days compared to the control, but were never on a biologically adverse level.
Bees intensively cleaning themselves, cramping bees and bees showing locomotion problems were observed particularly on the day of test item application.
The application of the reference item showed clear effects on the brood development, resulting in low brood indices, low compensation indices and high termination rates in two of the three replicates and high mortality values concerning pupae and larvae in all replicates of R.

Overall, based on the results of this study, Deltamethrin EW 15B G applied at a rate corresponding to 7.5 g a.s./ha does not adversely affect honey bee brood and colony development.

*****

Report:	**KCP 10.3.1.5/05, [redacted]; 2006**
Title:	Toxicity Testing of Deltamethrin EW 50 on Honey Bees (*Apis mellifera* L.) under Semi-Field Conditions - Tunnel Test.
Document No:	M-274120-01-1 (Rep. No.: 29011037)
Guidelines:	OEPP/EPPO (2001) Guideline for the efficacy evaluation of plant protection products – Side effects on honeybees. OEPP/EPPO, PP 1/170(3) update 2000; Revision (updated with ICPBR-recommendations) approved in 2000
GLP:	yes

~~**Material and Methods:**~~
~~Test item: Deltamethrin EW 50, Batch ID: 2005-004004, Sample Description: TOX07463-00, analysed content of AE F032640 (= deltamethrin): 49.7 g/L (4.78 % w/w) (nominal: 50 g/L).~~

~~A tunnel test was conducted, in order to assess the effect of Deltamethrin EW 50 on honey bees under semi-field conditions. Cages (14 m length × 5.5 m width × 2.5 m height) were set up on a 40 m² plot of flowering *Phacelia tanacetifolia* (2 × 20 m²) and small bee colonies were introduced 6 days before~~

the application. One bee hive was used per tunnel. The test item (7.5 g a.s. (156.9 g product) in 400 L water/ha), water (400 L water/ha) and a reference item (1.5 L Perfekthion EC (dimethoate) in 400 L water/ha) were applied on the whole plot of plants in two operations, with foraging bees present. The trial was performed using three tunnels for the test item treatment, the control and the reference item treatment (dimethoate 400 g/L), respectively. The total duration of the test was 7 days following the application.

Mortality and foraging activity (flight density) of the bees were assessed before and after application. Sublethal effects, such as changes in behaviour, were also monitored. Colony assessments (food stores, brood status and hive populations) were made twice, 2 days before the applications and at the end of the study (day + 7). Weather conditions were good during application. The sky was a little cloudy but warm with no precipitation. No rain occurred during the treatment day and the following 3 days. The weather was variable but warm for the remainder of the trial.

Findings:

Effect on honey bee mortality:

Starting conditions of the experiment were ideal, indicating similar natural mortality levels among the different treatment groups before application (no statistical significant difference of the hives, Dunnett`s t-test, multiple comparison to the control, two sided, $\alpha = 0.05$).

On the day of the test item application a short lasting and slight increase of bee mortality occurred, when a mean of 171.0 dead bees per colony were found in the test item treatment compared to 31.0 in the controls but this was not statistically significant compared to the control (Student t-test, one-sided greater, $\alpha = 0.05$). However, this level of mortality will not affect colony vitality or pollination activity of the colony.

The following day (day +1) mortality levels in the test item treatment remained slightly higher compared to the control, but this was not statistical significant anymore.

From day 2 onwards until the end of the assessment period on day 7, mortality levels of the bees after treatment with Deltamethrin EW 50 were comparable to the levels of the control treatments. At any day the number of dead bees per tunnel in the test item group did not differ from the control (Student t-test, pairwise comparison, $\alpha = 0.05$, one-sided greater). An overall comparison of the mean dead bees found in the traps and on the gauze did not show a statistical difference between the control and the test item treatment (Student t-test, pair-wise comparison to the control, one-sided greater, $\alpha = 0.05$).

After treatment with the reference item (dimethoate) a distinct increase of bee mortality was observed for the first five days. From day 1 to day 3 following the application the number of dead bees found in the reference item treatment was approximately 6 to 15 times higher compared to the control values, indicating the sensibility of the test system. An overall comparison of the mortality data indicates a statistically significant difference compared to the control (Student t-test, pairwise comparison to the control, one-sided greater, $\alpha = 0.05$).

Effects on honey bee flight intensity:

After application of Deltamethrin EW 50 flight intensity was reduced on the day of application (statistically significant differences, Welch t-test, pairwise comparison to the control, one-sided smaller, $\alpha = 0.05$). From day 1 onwards until the end of the trial the foraging activity of the bees were comparable or even higher in the test item treated tunnels compared to the controls. An overall comparison of the mean flight activity did not show a statistical difference between the control and the test item treatment (Welch t-test, pair-wise comparison to the control, one-sided smaller, $\alpha = 0.05$).

The foraging activity after application of the reference item (dimethoate) led to a clear decrease of flight intensity until the end of the experiment (7 days), which was statistically significant compared to the control on each single day (Welch t-test, pairwise comparison, one-sided smaller, $\alpha = 0.05$).

~~Effects on honey bee behaviour~~

~~Behavioural abnormalities *e.g.* poisoning symptoms such as discoordinated movement, apathy or an intensive cleaning behaviour were observed following the application after Deltamethrin EW 50 treatment on day 0 for ca. 4 hours. Up to a maximum of 65 bees per tunnel were observed with such symptoms. 6 hours following the application, these behavioural abnormalities had gone. On the next days until the end of the experiment no more behavioural impairments were noted at any time until test end in the test item treatment. No behavioural abnormalities could be observed in the control group. The reference item treatment caused behavioural abnormalities (moving coordination problems, abnormal cleaning) at least until the first day following the application of dimethoate.~~

~~Effects on honey bee brood development:~~

~~No adverse effect of the test item on the brood was observable. After the applications, all colonies showed a sufficient amount of all brood stages without any indication of a test item related effect. During the brood assessment 7 days following the application, all queens were found in the colonies.~~

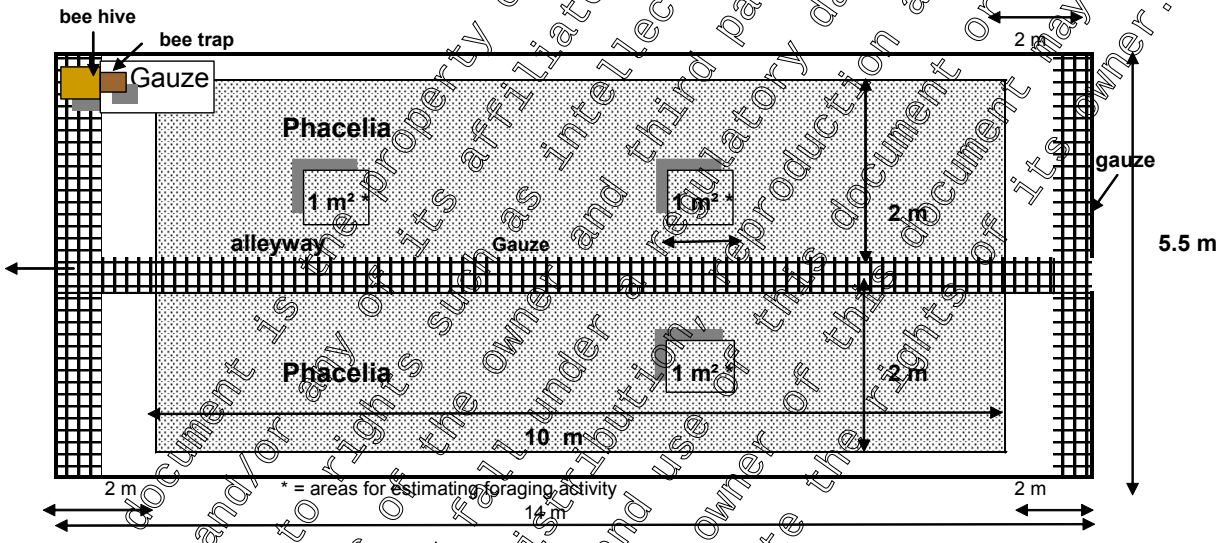
~~Conclusions~~

~~No ecologically relevant effects on mortality, flight intensity, behaviour or brood of the honey bees were observed after direct application of Deltamethrin EW 50 (7.5 g a.s./ha) in 400 L water/ha into a bee-attractive, flowering crop and during bee flight in a semi-field (tunnel) study. According to the results of this study, Deltamethrin EW 50 does not adversely affect honeybee colonies.~~

Material and Methods:

Test material	Deltamethrin EW 50
Test item:	Deltamethrin EW 50 content of a.s. (analysed): deltamethrin: 49.7 g/L, 4.78 % w/w (50 g a.s./L nominal).
Batch number:	2005-004004
Reference item:	Perfekthion EC (BAS 15211 I) (batch no.: 1810, 400 g a.s./L nominal, analysed content: 406.1 g a.s./L)
Test organism:	Honey bees (*Apis mellifera*)
Source:	Small honey bee colonies, maintained according to normal beekeeping practice [redacted]. No varroacide has been used in the hives for at least 2 month prior to the experimental start date. Healthy, well fed and queen-right colonies, containing about 5000 honey bees on 4 honeycombs with all brood stages present were used for the study.
Crop:	*Phacelia tanacetifolia,* Type: Boratus, at flowering (height: ca. 30 - 50 cm)
Test location:	[redacted]

Test unit: Size of the tunnels: 14 m length × 5.5 m width × 2.5 m height, tunnels were semi-circular in cross-section and constructed out of a tubular steel frame, covered with synthetic gauze (mesh size ca. 2 mm) and were placed on the crops a few days before experimental start with a distance of *ca.* 2 meters in between. The tunnels were placed over the flowering plants a few days before experimental starting date. One small bee hive, a trap for collecting dead bees and one drinking trough were installed in each tunnel. In order to facilitate the collection of dead bees, the plants on both ends of the tunnels were removed, thereby creating bare paths on the ground of approximately 0.5 m width and covered by gauze strips (see test design layout in each tent).



Application rates: Control (C): Tap water
Treatment rate (T): 7.5 g a.s./ha during foraging activity
Reference rate (R): 1.5 L/ha (600 g a.s./ha)
Three replicates per treatment group were used.
The spray volume was 400 L/ha in all treatment groups. The sprayer was calibrated before use. The deviation reached a maximum of 0.6%.

Data analysis: Mortality and flight density data were tested for normal distribution using R's test or Kolmogorroff/Smirnov test and homogeneity of variance using Cochran`s test.
Before Application: a multiple and two-sided comparison ($\alpha = 0.05$) was done for the comparison of the mortality and flight density data before application using Dunnett`s t-test for homogeneous variances.
After Application: a pairwise comparison ($\alpha = 0.05$) was done for the comparison of the mortality and flight density data after application using Student t-test for homogeneous variances (mortality) or Welch t-test for inhomogeneous variances (flight density).
The computer program used to perform the statistical analysis was TOX Rat® Pro version 2.09, Spirit Solutions (2005).

Deviations from the study plan: No deviations to the study plan occurred.

Climatic conditions during the experiment:

The day of application and the following days of the experiment were characterized by sunny and warm summer weather, which resulted in a high foraging activity of the bees. On day 4 and 5 following the application some rain occurred with a precipitation of 5.4 and 4.4 mm. This precipitation occurred during the evening or the night on these days. The mean day temperature following the application was between 16.8 and 23.2°C. The environmental conditions during the whole experiment are shown in the table below.

Table 1: Data on the climatic conditions during the experiment

day	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7
rain [mm][1]	0.0	0.0	0.0	14.4	0.0	0.0	0.0	0.0	0.0	5.4	4.4	0.0	0.0
temperature [°C][2]	18.9	17.7	21.5	23.0	20.5	21.7	18.5	16.8	19.5	23.2	20.8	21.7	18.2
minmum [°C]	14.9	9.5	11.2	16.6	14.7	18.7	14.6	11.6	10.8	16.5	16.8	15.1	14.0
maximum [°C]	24.0	24.9	29.6	28.6	25.6	25.6	21.3	22.5	27.8	31.9	26.5	26.5	24.8
rel humidity [%][2]	81.9	70.6	68.1	79.3	87.6	76.4	68.0	64.4	74.0	79.6	80.5	74.5	88.4

[1] total precipitation per day

[2] daily mean values

day = days in relation to the application day (= day 0)

Pesticide history of the field site:

Management of the Field (non-GLP): *Phacelia tanacetifolia* until August 2005, afterwards rape was used as an intercrop between 2005 and the seeding of the new *Phacelia*.

Pesticides and Fertilisation (non-GLP): Horse manure (180 dt/ha) was spread on the field in 2005 and the area was treated with 2.0 L/ha Butisan Top (Herbicide, 375 g/L Metazachlor, 125 g/L Quinmerac, non-toxic to bees, class B4).

The effects of 'Deltamethrin EW 50' were tested on the honeybee (*Apis mellifera* L.) under semi-field conditions in a tunnel test.

Honey bees (*A. mellifera* L.) can be affected by pesticide residues on plants, via oral intake with contaminated food or water and/or by direct contact in the course of application during normal farming practice.

Investigations under semi-field conditions serve as practical tests to estimate the effects of Deltamethrin EW 50 to honey bees in tunnels under full field conditions.

Mortality and foraging activity of the bees were assessed before and after application. Sublethal effects, such as changes in behaviour, were also monitored. Colony assessments (food stores, brood status) were made at the beginning and at the end of the study. Results for the test item were compared with those for the control while the results for the reference item were used to demonstrate exposure of the bees under the test conditions.

The following endpoints were assessed:

- Mortality: Assessment of dead bees from the gauze strips in the tunnels (laid on the bare ground in front of the hive and at both ends of the tunnels) and from dead-bee traps placed in front of each hive at different time point before and after the application in the control, the test item group and the reference item group, respectively
- Flight intensity (number of bees foraging on flower plants/m^2) in each tunnel before and after the applications in the control, the test item group and the reference item group, respectively
- Behaviour abnormalities of the bees at the hive entrance or on the plants like e.g. intensive cleaning, restless or moving coordination problems were recorded.
- State of brood (occurrence of eggs, young and old larvae) and supply of pollen and food in the colonies were estimated once before (2DBT) and once after the experimental observation time (7DAT) following the application. This was carried out by estimating the area of each frame (in percentage of the total area = both sides of the frame) containing brood and/or food stores in the control, the test item group and the reference item group, respectively.

Dates of Work: 16th June to 28th June 2006

Findings:

Honey Bee Mortality

Data on mortality of the bees in the water treated control tunnels, in the Deltamethrin EW treated tunnels and in tunnels treated with the reference item are given in the following tables.

Table 2: Summarised mortality data

Time	Water treated control			Deltamethrin EW 50 @7.5 g a.s./ha				Reference Item			
	dead bees			dead bees				dead bees			
	total[b]	mean[c]	SD	total[b]	mean[c]	SD	Statistic	total[b]	mean[c]	SD	Statistic
5DBT	30	10	± 6.2	19	6.3	± 0.6	-	19	6.3	± 4.5	-
4DBT	86	28.7	± 27.0	96	32	± 4.6	-	111	37	± 5.6	-
3DBT	93	31	± 24.3	103	34.3	± 12.7	-	143	47.7	± 26.3	-
2DBT	162	54	± 47.7	197	65.7	± 8.3	-	154	51.3	± 21.4	-
1DBT	96	32	± 31.4	96	32	± 14.0	-	178	59.3	± 12.9	-
0DBT	119	39.7	± 35.5	154	51.3	± 31.4	-	175	58.3	± 3.5	-
Daily mean 5DBT to 0DBT	98	32.6	± 14.4	111	36.9	± 20.0	n.s.	130	43.3	± 19.9	n.s.
0DAT	93	31	± 25.9	513	171	± 32.2	n.s.	1177	392.3	± 152.4	*
1DAT	57	19	± 19.0	170	56.7	± 11.0	n.s.	848	282.7	± 109.8	*
2DAT	67	22.3	± 16.5	77	25.7	± 7.5	n.s.	387	129	± 36.4	*
3DAT	171	57	± 49.6	121	40.3	± 15.7	n.s.	675	225	± 94.0	*
4DAT	150	50	± 38.6	128	42.7	± 24.7	n.s.	361	120.3	± 34.6	*
5DAT	117	39	± 23.4	144	48	± 15.7	n.s.	301	100.3	± 18.5	*
6DAT	208	69.3	± 57.0	287	95.7	± 23.5	n.s.	331	110.3	± 71.8	n.s.
7DAT	57	19	± 14.7	84	28	± 2.0	n.s.	108	36	± 6.6	*
Daily mean 0DAT to 7DAT	115	38.3	± 18.9	191	63.5	± 48.6	n.s.	524	174.5	± 116.8	*

DBT Days before treatment
DAT Days after treatment
n.s. Not statistical significant from the control;
* Statistical significant from the control
b Total number of three tunnels of each treatment group
c Mean values (rounded) of three tunnels per treatment group
Statistic: Dunnett's t-test, multiple (before application); Student t-test, one-sided greater (after application), a = 0.05

Table 3: Mortality of the bees in the water treated control tunnels

Water control	Number of dead bees on the gauze						Number of dead bees in the dead bee traps						Dead bees		
	Tunnel no.:			Total	mean[b]	sd	Tunnel no.:			Total	mean[b]	sd			
Time	1	2	3				1	2	3				Total	mean[b]	sd
5DBT	7	14	4	25	8.3	5.1	1	3	1	5	1.7	1.2	30	10.0	6.2
4DBT	58	23	3	84	28.0	27.8	0	0	2	2	0.7	1.2	86	28.7	27.0
3DBT	46	41	3	90	30.0	23.5	0	3	0	3	1.0	1.7	93	31.0	24.3
2DBT	95	57	2	154	51.3	46.8	4	2	2	8	2.7	1.2	162	54.0	47.7
1DBT	61	24	4	89	29.7	28.9	5	2	0	7	2.3	2.5	96	32.0	31.4
0DBT															
09:40	63	23	6	92	30.7	29.3	3	0	1	4	1.3	1.5	96	32.0	30.5
13:50	12	10	1	23	7.7	5.9	0	0	0	0	0.0	0	23	7.7	5.9
Total 0DBT	75	33	7	115	38.3	34.3	3	0	1	4	1.3	1.5	119	39.7	35.5
14:10 a.a.	17	4	0	21	7.0	8.9	0	0	0	0	0.0	0	21	7.0	8.9
15:10	4	3	0	7	2.3	2.1	0	0	0	0	0.0	0	7	2.3	2.1
17:10	26	9	5	40	13.3	11.2	0	0	0	0	0.0	0	40	13.3	11.2
19:10	12	9	3	24	8.0	4.6	0	1	0	1	0.3	0.6	25	8.3	4.7
Total 0DAT	59	25	8	92	30.7	26.0	0	1	0	1	0.3	0.6	93	31.0	25.9
1DAT															
10:10	21	8	1	30	10.0	10.1	1	1	0	2	0.7	0.6	32	10.7	10.6
14:30	17	5	2	24	8.0	7.9	1	0	0	1	0.3	0.6	25	8.3	8.5
Total 1DAT	38	13	3	54	18.0	18.0	2	1	0	3	1.0	1	57	19.0	19.0
2DAT	38	22	6	66	22.0	16.0	1	0	0	1	0.3	0.6	67	22.3	16.5
3DAT	102	60	5	167	55.7	48.6	3	0	1	4	1.3	1.5	171	57.0	49.6
4DAT	77	63	6	146	48.7	37.6	1	3	0	4	1.3	1.5	150	50.0	38.6
5DAT	50	52	7	109	36.3	25.4	1	2	5	8	2.7	2.1	117	39.0	23.4
6DAT	117	64	9	200	66.7	59.0	0	4	4	8	2.7	2.3	208	69.3	57.0
7DAT	34	15	3	52	17.3	15.6	1	1	3	5	1.7	1.2	57	19.0	14.7

DBT Days before treatment
DAT Days after treatment
a.a. After application

sd Standard deviation
[b] Mean values of dead bees per tunnel

Table 4: Mortality of the bees in the Deltamethrin EW 50 treated tunnels at 7.5 g a.s./ha

Deltamethrin EW 50 @7.5 g a.s./ha	Number of dead bees on the gauze						Number of dead bees in the dead bee traps						Dead bees		
	Tunnel no.:			Total	mean[a]	sd	Tunnel no.:			Total	mean[a]	sd			
Time[a]	1	2	3				1	2	3				Total	mean[b]	sd
5DBT	6	7	5	18	6.0	1.0	0	0	1	1	0.3	0.6	19	6.3	0.6
4DBT	37	30	27	94	31.3	5.1	0	1	1	2	0.7	0.6	96	32.0	4.6
3DBT	48	26	25	99	33.0	13.0	1	1	2	4	1.3	0.6	103	34.3	12.7
2DBT	74	61	54	189	63.0	10.1	1	2	5	8	2.7	2.1	197	65.7	8.3
1DBT	45	32	18	95	31.7	13.5	1	0	0	1	0.3	0.6	96	32.0	14.0
0DBT															
09:40	49	21	16	86	28.7	17.8	3	2	3	8	2.7	0.6	94	31.3	18.0
13:50	34	16	9	59	19.7	12.9	1	0	0	1	0.3	0.6	60	20.0	13.5
Total 0DBT	83	37	25	145	48.3	30.6	4	2	3	9	3.0	1.0	154	51.3	31.4
14:10 a.a.	62	32	14	108	36.0	24.2	0	0	0	0	0.0	0.0	108	36.0	24.2
15:10	52	28	67	147	49.0	19.7	0	0	0	0	0.0	0.0	147	49.0	19.7
17:10	45	71	49	165	55.0	14.0	0	2	0	2	0.7	1.2	167	55.7	15.1
19:10	46	34	11	91	30.3	17.8	0	0	0	0	0.0	0.0	91	30.3	17.8
Total 0DAT	205	165	141	511	170.3	32.3	0	2	0	2	0.7	1.2	513	171.0	32.2
1DAT															
10:10	32	37	45	114	38.0	6.6	4	1	1	6	2.0	1.7	120	40.0	5.3
14:30	9	15	23	47	15.7	7.0	3	0	0	3	1.0	1.7	50	16.7	5.7
Total 1DAT	41	52	68	161	53.7	13.6	7	1	1	9	3.0	3.5	170	56.7	11.0
2DAT	16	26	33	75	25.0	8.5	2	0	0	2	0.7	1.2	77	25.7	7.5
3DAT	58	35	28	121	40.3	15.7	0	0	0	0	0.0	0.0	121	40.3	15.7
4DAT	38	66	20	124	41.3	23.2	1	3	0	4	1.3	1.5	128	42.7	24.7
5DAT	43	83	34	140	46.7	14.8	2	2	0	4	1.3	1.2	144	48.0	15.7
6DAT	71	118	96	285	95.0	23.5	1	1	0	2	0.7	0.6	287	95.7	23.5
7DAT	26	28	30	84	28.0	2.0.	0	0	0	0	0.0	0.0	84	28.0	2.0

DBT Days before treatment
DAT Days after treatment
a.a. After application
sd Standard deviation
[b] Mean values of dead bees per tunnel

Table 5: Mortality of the bees in the reference item treated tunnels

Reference item	Number of dead bees on the gauze						Number of dead bees in the dead bee traps						Dead bees		
	Tunnel no.:			Total	mean[a]	sd	Tunnel no.:			Total	mean[a]	sd	Total	mean[b]	sd
Time[a]	1	2	3				1	2	3						
5DBT	10	6	2	18	6.0	4.0	1	0	0	1	0.3	0.6	19	6.3	4.5
4DBT	41	37	31	109	36.3	5.0	1	1	0	2	0.7	0.6	111	37.0	5.6
3DBT	76	40	22	138	46.0	27.5	0	3	2	5	1.7	1.5	143	47.7	26.3
2DBT	76	39	37	152	50.7	22.0	0	0	2	2	0.7	1.2	154	51.3	21.4
1DBT	59	56	42	157	52.3	9.1	4	14	3	21	7.0	6.1	178	59.3	12.9
0DBT															
09:40	36	25	36	97	32.3	6.4	0	2	2	4	1.3	1.2	101	33.7	5.9
13:50	26	24	17	67	22.3	4.7	0	7	0	7	2.3	4.0	74	24.7	7.1
Total 0DBT	62	49	53	164	54.7	6.7	0	9	2	11	3.7	4.7	175	58.3	3.5
14:10 a.a.	93	62	102	257	85.7	21.0	3	18	17	38	12.7	8.4	295	98.3	19.6
15:10	65	53	37	155	51.7	14.0	24	32	52	108	36.0	14.4	263	87.7	2.3
17:10	67	17	103	187	62.3	43.2	32	15	75	122	40.7	30.8	309	103.0	73.1
19:10	44	25	85	154	51.3	30.7	51	25	80	156	52.0	27.5	310	103.3	58.0
Total 0DAT	269	157	327	753	251.0	86.4	110	90	224	424	141.3	72.3	1177	392.3	152.4
1DAT															
10:10	100	34	106	240	80.0	39.9	110	78	184	372	124.0	54.4	612	204.0	89.2
14:30	63	17	28	108	36.0	24.0	78	27	23	128	42.7	30.7	236	78.7	54.1
Total 1DAT	163	51	134	348	116.0	58.1	188	105	207	500	166.7	54.2	848	282.7	109.8
2DAT	82	33	61	176	58.7	24.6	89	73	49	211	70.3	20.1	387	129.0	36.4
3DAT	184	71	266	521	173.7	97.9	63	51	40	154	51.3	11.5	675	225.0	94.0
4DAT	97	73	100	270	90.0	14.8	49	8	34	91	30.3	20.7	361	120.3	34.6
5DAT	69	68	90	227	75.7	12.4	50	14	10	74	24.7	22.0	301	100.3	18.5
6DAT	129	23	139	291	97.0	64.3	31	5	4	40	13.3	15.3	331	110.3	1.8
7DAT	34	33	26	93	31.0	4.4	9	2	4	15	5.0	3.6	108	36.0	6.6

DBT Days before treatment
DAT Days after treatment
a.a. After application
sd Standard deviation
[b] Mean values of dead bees per tunnel

Before application there was no statistically significant difference in mortality between the treatment groups. On the day of application mortality rates were slightly higher in the test item group (171.0) compared to the control (31.0), but the number of dead bees found on the day of application in the test item treated group was not statistically significant increased compared to the control (Student t-test, pairwise comparison, $\alpha = 0.05$, one-sided greater). From day 2 (2DAT) onwards until the end of the experiment (7DAT) no statistical significant differences between the test item treatment and the control could be observed (Student t-test, pairwise comparison, $\alpha = 0.05$, one-sided greater).

In contrast to the observations in the test item treatment group and the control group, application of the reference item (dimethoate at a rate of 600 g a.i./ha) resulted in a marked and increased number of dead bees found in the traps and on the strips between day 0 and day 5, which was statistically significant different from the control (Student t-test, pairwise comparison, $\alpha = 0.05$, one-sided greater). Mortality increased up to *ca.* 13x the levels of the control values directly after application.

Honey Bee Flight Intensity

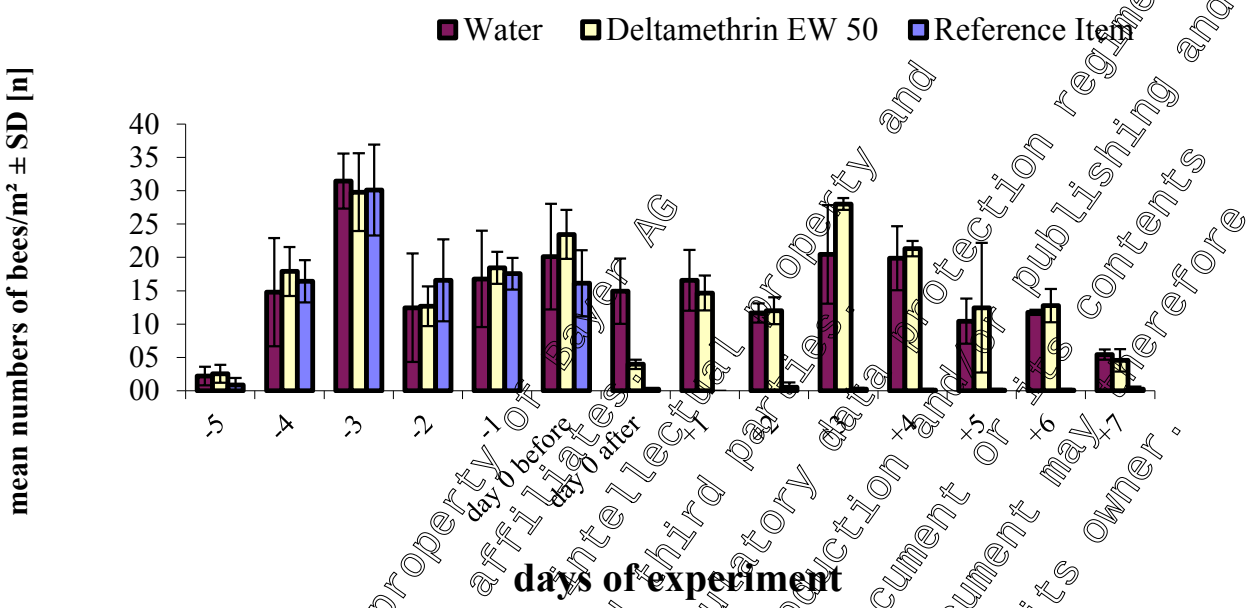
A summary of the honey bee flight intensity results is shown in the table below.

Table 6: Summarized flight density data

time[a]	water treated control mean number of bees per m² [b]		Deltamethrin EW 50 mean number of bees per m² [b]		statistics	Reference Item mean number of bees per m² [b]		statistics
day -5	2.2	± 1.4	2.6	± 1.3	-	0.9	± 1.0	-
day -4	14.8	± 8.1	17.9	± 3.7	-	16.4	± 3.2	-
day -3	31.4	± 4.1	29.8	± 5.8	-	30.1	± 6.8	-
day -2	12.4	± 8.1	12.7	± 3.0	-	16.6	± 6.1	-
day -1	16.8	± 7.2	18.4	± 2.4	-	17.6	± 2.4	-
mean day 0 b.a.[c]	**20.1**	**± 7.9**	**23.4**	**± 3.7**	**-**	**16.1**	**± 4.9**	**-**
daily mean day -5 to 0 b.a.	**16.3**	**± 9.6**	**17.5**	**± 9.3**	**n.s.**	**16.3**	**± 9.3**	**n.s.**
mean day 0 a.a.[d]	**14.9**	**± 4.9**	**4.0**	**± 0.7**	*****	**0.2**	**± 0.2**	*****
day 1	10.6	± 4.6	14.2	± 2.6	n.s.	0.0	± 0.0	*
day 2	11.7	± 1.5	12.0	± 2.0	n.s.	0.6	± 0.7	*
day 3	20.4	± 7.4	28.0	± 0.9	n.s.	0.2	± 0.2	*
day 4	19.9	± 4.8	21.3	± 1.2	n.s.	0.1	± 0.2	*
day 5	10.4	± 3.4	12.4	± 9.7	n.s.	0.1	± 0.2	*
day 6	11.8	± 0.4	12.8	± 2.5	n.s.	0.1	± 0.2	*
day 7	3.4	± 0.8	4.6	± 1.7	n.s.	0.2	± 0.4	*
daily mean day 0 a.a. to day 7	**13.9**	**± 5.1**	**13.7**	**± 8.0**	**n.s.**	**0.2**	**± 0.2**	*****

[a] days -5 to -1 = days before application; day 0 = application day; day 1 to 7 = days after application
[b] mean values (rounded) of three tunnels per treatment group
[c] b.a.= before application; [d] a.a.= after application
n.s. = not statistical significant to the control; * = statistical significant to the control
Statistic: Dunnett`s t-test, multiple (before application); $\alpha = 0.05$
Welch test, one-sided smaller (after application) / $\alpha = 0.05$

Table 7: Flight density of the honey bees during the experiment



Mean number of foraging bees of three tunnels per treatment group; day 0 before = before application; day 0 after = after application.

Between 5DBT and 1DBT the mean flight densities among the colonies ranged from 2.2 to 31.4 bees per m² in the control, 2.6 to 29.8 bees per m² in the test item group and 0.9 to 30.1 bees per m² in the reference item. No statistically significant difference in flight intensity was found between the colonies of the overall daily mean of this period.

Following the test item application, foraging activities decreased until the end of the application day (statistical difference to control: Welch t-test, pairwise comparison, $\alpha = 0.05$, one-sided smaller). This was not observed in the control tunnels. Flight intensities on the following days (1DAT to 7DAT) were comparable or even higher in the test item treated tunnels compared to the control tunnels. There was no statistical significant difference detectable compared to the control for each assessment day (Welch t-test, pairwise comparison, $\alpha = 0.05$, one-sided smaller).

After treatment of the reference item (dimethoate) there was a fairly rapid and significant reduction in flight intensity. Shortly after application, the bees returned to the hive so that about one hour after application, average flight activity was 2 % of the pre-application level and within two hours it was down to about 0 %. Flight intensity remained very low for the remainder of the trial and only a few bees were seen foraging on the flowers over the next 7 days.

Behaviour of the Bees

Following the test item application up to maximum 65 bees per tunnel showed some behavioural impairments like discoordinated movement, apathy, cramps or an intensive cleaning behaviour, but this was gone until the evening of the application day and was not seen any more until the end of the experiment. No behavioural abnormalities could be observed in the control group.

The reference item treatment caused also behavioural abnormalities (moving coordination problems and intensive cleaning) at least until the first day following the application of dimethoate.

Check of Brood

Table 8: Brood estimation of the colonies 2 days before the applications

water treatment (control)

	tunnel no.:1 honeycomb no.:				tunnel no.: 2 honeycomb no.:				tunnel no.: 3 honeycomb no.:			
	1	2	3	4	1	2	3	4	1	2	3	4
nectar [a]	0%	15%	15%	65%	25%	15%	30%	0%	90%	20%	15%	0%
pollen [a]	0%	0%	20%	30%	20%	10%	5%	0%	0%	10%	5%	0%
eggs [a]	0%	20%	20%	0%	0%	20%	5%	0%	0%	15%	30%	0%
maggots [a]	0%	5%	20%	0%	5%	35%	10%	0%	0%	20%	30%	0%
closed brood [a]	0%	35%	10%	0%	50%	20%	40%	0%	0%	35%	20%	0%
empty areas [a]	100%	25%	15%	5%	0%	0%	10%	100%	10%	0%	0%	100%

Deltamethrin EW 50

	tunnel no.: 1 honeycomb no.:				tunnel no.: 2 honeycomb no.:				tunnel.: 3 honeycomb no.:			
	1	2	3	4	1	2	3	4	1	2	3	4
nectar [a]	0%	10%	20%	30%	15%	0%	0%	25%	20%	10%	20%	80%
pollen [a]	0%	10%	5%	30%	5%	0%	0%	30%	0%	0%	10%	10%
eggs [a]	0%	15%	15%	15%	0%	20%	5%	5%	20%	15%	25%	5%
maggots [a]	0%	20%	5%	20%	20%	0%	0%	10%	0%	15%	35%	5%
closed brood [a]	0%	40%	50%	5%	50%	0%	65%	30%	60%	20%	10%	0%
empty areas [a]	100%	5%	15%	0%	10%	30%	30%	0%	0%	40%	0%	0%

Reference Item treatment

	tunnel no.: 1 honeycomb no.:				tunnel no.: 2 honeycomb no.:				tunnel no.: 3 honeycomb no.:			
	1	2	3	4	1	2	3	4	1	2	3	4
nectar [a]	10%	20%	20%	70%	50%	5%	20%	5%	0%	10%	10%	40%
pollen [a]	0%	10%	20%	5%	30%	5%	15%	0%	0%	0%	20%	20%
eggs [a]	0%	10%	20%	0%	0%	0%	20%	15%	10%	10%	20%	15%
maggots [a]	0%	45%	30%	0%	0%	0%	20%	10%	0%	20%	20%	15%
closed brood [a]	0%	15%	20%	20%	0%	0%	20%	30%	0%	60%	30%	10%
empty areas [a]	90%	0%	0%	5%	20%	90%	5%	40%	90%	0%	0%	0%

[a] amount of brood/nectar/pollen estimated in percentage of the whole comb (both sides)

Table 9: Brood estimation of the colonies 7 days after the applications

	water treatment (control)											
	tunnel no.:1 honeycomb no.:				**tunnel no.: 2** honeycomb no.:				**tunnel no.: 3** honeycomb no.:			
	1	2	3	4	1	2	3	4	1	2	3	4
nectar [a]	0%	30%	20%	50%	25%	30%	30%	15%	85%	25%	35%	15%
pollen [a]	0%	0%	25%	50%	15%	10%	0%	0%	0%	15%	0%	5%
eggs [a]	0%	5%	0%	0%	15%	10%	20%	0%	0%	15%	15%	0%
maggots [a]	0%	5%	20%	0%	15%	10%	0%	0%	0%	10%	15%	0%
closed brood [a]	0%	40%	35%	0%	30%	40%	30%	0%	0%	35%	35%	0%
empty areas [a]	100%	20%	0%	0%	0%	0%	20%	85%	15%	0%	0%	80%

	Deltamethrin EW 50											
	tunnel no.: 1 honeycomb no.:				**tunnel no.: 2** honeycomb no.:				**tunnel.: 3** honeycomb no.:			
	1	2	3	4	1	2	3	4	1	2	3	4
nectar [a]	10%	5%	25%	30%	25%	15%	35%	30%	50%	30%	20%	70%
pollen [a]	0%	0%	0%	15%	5%	0%	10%	25%	5%	5%	5%	0%
eggs [a]	0%	5%	20%	0%	10%	20%	0%	0%	0%	0%	0%	10%
maggots [a]	0%	0%	25%	0%	20%	0%	30%	0%	15%	0%	5%	0%
closed brood [a]	0%	80%	30%	20%	30%	30%	25%	25%	10%	45%	50%	5%
empty areas [a]	90%	10%	0%	35%	10%	35%	0%	20%	20%	20%	20%	15%

	Reference Item treatment											
	tunnel no.: 1 honeycomb no.:				**tunnel no.: 2** honeycomb no.:				**tunnel no.: 3** honeycomb no.:			
	1	2	3	4	1	2	3	4	1	2	3	4
nectar [a]	20%	15%	40%	35%	70%	0%	25%	15%	0%	25%	20%	35%
pollen [a]	0%	5%	10%	10%	20%	5%	5%	0%	0%	5%	15%	30%
eggs [a]	0%	15%	10%	0%	0%	55%	0%	0%	0%	10%	10%	0%
maggots [a]	0%	0%	15%	0%	0%	0%	25%	0%	0%	0%	10%	0%
closed brood [a]	0%	50%	20%	30%	0%	20%	30%	50%	0%	40%	25%	20%
empty areas	80%	15%	5%	25%	10%	20%	15%	35%	100%	20%	20%	15%

[a] amount of brood/nectar/pollen estimated in percentage of the whole comb (both sides)

Two days before and seven days following the application, a check of the brood was conducted in order to assess possible effects of the test item on colony development.

All stages of brood (eggs, larvae and closed brood) were found during the pre-application check in all colonies of all tunnels. As well a sufficient number of food and pollen was found as an indication of normal behaviour.

Seven days following the applications all brood stages could be found at the end of the test in each of the colonies. Furthermore, in each colony among the treatment groups an alive queen could be found indicating healthy colonies.

Conclusion:

Deltamethrin EW 50 was applied at a rate corresponding to 7.5 g a.s./ha during flowering to the highly bee-attractive crop *Phacelia tanacetifolia* with honey bees actively foraging on the crop during application. The effects on bee hives under confined exposure conditions considering mortality, flight intensity, behaviour, brood development were evaluated.

The mortality values in the test item treatment group during the post-application phase were statistically not different compared to the control.

Following the test item application, foraging activities decreased until the end of the application day (statistical difference to control: Welch t-test, pairwise comparison, $\alpha = 0.05$, one-sided smaller). Flight intensities on the following days (1DAT to 7DAT) were comparable or even higher in the test item treated tunnels compared to the control tunnels.

Following the test item application up to maximum 65 bees per tunnel showed some behavioural impairments like discoordinated movement, apathy, cramps or an intensive cleaning behaviour, but this was gone until the evening of the application day and was not seen any more until the end of the experiment. No behavioural abnormalities could be observed in the control group.

Seven days following the applications all brood stages could be found at the end of the test in each of the colonies. Furthermore, in each colony among the treatment groups a healthy queen could be found indicating healthy colonies.

Overall no ecologically relevant effects on mortality, flight intensity, behaviour or brood of the honey bees were observed after direct application of Deltamethrin EW 50 (7.5 g a.s./ha) in 400 L water/ha into a bee-attractive, flowering crop and during bee flight in a semi-field (tunnel) study. According to the results of this study Deltamethrin EW 50 does not adversely affect honeybee colonies.

*****

Report:	**KCP 10.3.1.5/06, [redacted], 2001**
Title:	Assessment of Side Effects of AE F032640 00 EC02 A804 on the Honey bee (*Apis mellifera* L.) in the Semi-Field
Document No:	M-200492-01-1 (Rep. No.: 20001132/01-BZEU)
Guidelines:	EPPO 170
GLP:	yes

~~**Material and Methods:**~~

~~The side effects of the test substance AE F032640 00 EC02 A804 were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions according to the guideline of the European and Mediterranean Plant Protection Organization No. 170 (EPPO, 1992). The test substance AE F032640 00 EC02 A804 was applied at an application rate of 7.5 g a.i./ha in 300 L water/ha. Plots treated with tap water served as control. As toxic standard, Hostathion 40 EC was applied at a concentration of 0.6 L/ha in 300 L water/ha. The effect of the test substance was examined on small bee colonies in cages placed over plots with flowering *Phacelia tanacetifolia* Benth. The influence of AE F032640 00 EC02 A804 was evaluated by comparing the effect of the test substance treatment group to the effect of the control group and toxic standard group regarding the following observations:~~

- ~~Mortality at the edge of the treated area and in the bee traps~~

- Foraging activity (number of forager bees/m^2 flowering *Phacelia* crop)
- Behaviour of the bees on the crop and around the hive
- Development of the bee brood

Findings and Conclusion:

Effect on honey bee mortality:

The application of the test substance AE F032640 00 EC02 A804 resulted in an increase of the mortality restricted to the day of application DAA 0aa (133.7 dead bees/colony) which was determined to be not significantly different to the control (74.3 dead bees/colony). A drastically increase of mortality was observed after application of the toxic standard with an average of 536.7 dead bees/colony. The effect of the toxic standard demonstrated the sensitivity of the method in detecting the toxic effects of a pesticide. When comparing the average pre-application mortality and the average postapplication mortality utilising $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality) no increase of mortality occurred after application of the test substance AE F032640 00 EC02 A804. The values for $Q_{M(average)}$ were calculated as 1.0 in the test substance treatment group and 0.7 in the control group. The value for $Q_{M(average)}$ in the toxic standard treatment was determined as 4.6.

Effects on honey bee flight intensity:

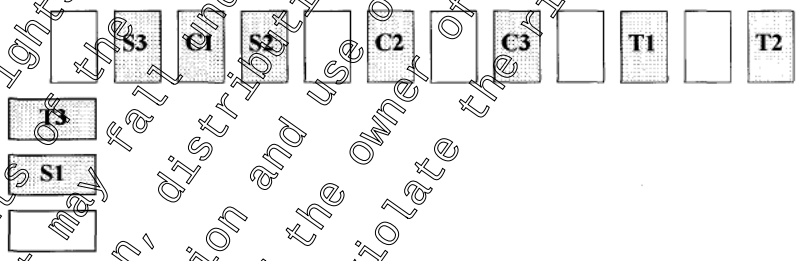
In the AE F032640 00 EC02 A804 treatment group an obvious repellent effect occurred directly after application assumed by the behaviour of the bees and confirmed due to the flight intensity (9.2 bees/m^2) on this day which remained significantly below the level of the control group (23.2 bees/m^2). The significantly reduced flight intensity in the AE F032640 00 EC02 A804 treatment group and in the toxic standard treatment lasted until evaluation day DAA1. Compared with the pre-application period the average daily post-application level of flight intensity was lower in the test substance treatment group AE F032640 00 EC02 A804 and in the toxic standard treatment but higher in the control group.

Effects on honey bee brood development:

Regarding the colonies strength and the bee brood development no abnormal differences attributable to the influence of the test substance were observed between the test substance groups and control.

Material and Methods:

Test material:	Deltamethrin EC 15
Test item:	AE F032640 00 EC02 A804 content of a.s. (analysed): deltamethrin: 1.66 % w/w (15.0 g a.s./L nominal).
Batch number:	TA15199PM
Reference item:	Hostathion 40 EC (active ingredient: triazophos; content of a.s. (analysed): 40.9 % (40 % nominal)
Test organism:	Honey bees (*Apis mellifera*) For the test, small healthy colonies with at least three combs (size of the combs ("Zandermaß"): 420 mm x 220 mm) were used. All nuclei were produced at the same time. The corresponding queens originated from one breeding line in order to guarantee uniform bee material in all treatment groups. Furthermore the following criteria for the nuclei were guaranteed:

	• at least two brood combs containing eggs, larvae and capped cells • at least one honey and pollen comb • bees are free of Nosema and other bee diseases Wooden bee traps (35 cm x 35 cm) with gauze on bottom and on 50 % of the top were attached to the entrance of the nucleus in order to register those dead bees which are carried out of the hives.
Source:	Not stated in the report.
Crop:	*Phacelia tanacetifolia* (bee attractive crop) was full in bloom
Test location:	The semi-field test was located in the south of Germany [redacted]
Test unit:	The dimensions of the floor of the tents were 4.8 m x 3.6 m and the height was 2 m. The tent frames were covered with light plastic gauze. The tents were placed over the plots of flowering Phacelia. A path of approx. 0.6 m was left at each side between the plots and the tent walls. The path was covered with linen sheet. The size of each plot covered with Phacelia tanacetifolia was approximately 8 m². Arrangement of the different variants during the semi-field test is shown below.  T = Toxic standard S = Test substance Deltamethrin EC 15 (AE F032640 00 EC02 A804) C = Control
Application rates:	Control (C): drinking water Treatment rate (T): 7.5 g a.s./ha Reference item (R): 0.6 L formulation/ha (240 g a.s./ha) Three replicates per treatment group referred to in this summary as: colony1, colony2 and colony3. The spray volume was 300 L/ha in all treatment groups.
Data analysis:	The influence of the test substances Deltamethrin EC 15 was evaluated by comparing the bees in the test cage to the control bees treated with water and those treated with the toxic standard and furthermore by comparing the pre- and post-application results in view of the following observations: • Mortality at the edge of the treated area and in the bee traps

- Foraging activity (number of forager bees/m^2 flowering *Phacelia* crop)
- Behaviour of the bees on the crop and around the hive
- Development of the bee brood

Deviations from the study plan: A number of deviations were recorded in the study report. As these were procedural deviations and do not impact the study results they have not been reported. However, in the control hive no.3 there were no eggs and larval stages observed at the brood assessment 2 days prior to the application. Therefore this hive was not taken into consideration

Climatic conditions during the experiment:

The environmental conditions are shown in table below.

Table 1: Weather conditions during the trial; temperature and precipitation were provided by GAB weather station in Niefern

Date	DAA	Ø Temperature min/max [°C]	Precipitation [mm]	Cloud formation at time of evaluation [%]
30JUN2000	- 6	7.9/23.7	0.0	0
01JUL2000	- 5	10.3/29.1	0.0	0
02JUL2000	- 4	15.0/28.8	7.6	80
03JUL2000	- 3	16.8/25.1	5.8	100
04JUL2000	- 2	14.3/23.3	14.4	100
05JUL2000	- 1	11.9/22.0	2.4	50
06JUL2000	0	10.9/26.0	0.2	0-5
07JUL2000	1	14.6/25.4	7.0	15-30
08JUL2000	2	10.8/16.7	13.6	100
09JUL2000	3	10.6/18.1	2.2	100
10JUL2000	4	12.9/19.9	12.4	100
11JUL2000	5	12.2/18.7	0.0	70-100

Pesticide history of the field site:

The pesticide history of the field site was not stated in the report.

The side effects of the test substance Deltamethrin EC 15 were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the EPPO guideline No. 170 (EPPO, 1992).

This study included three exposure groups with three replicates (tents) each: one drinking water treated control group (C), one test-item groups (T) and one reference item group (R). The hives were introduced into the test cages 7 days before the application of the test substance to enable the bees getting familiar with the environment and to lower the mortality which usually is increased due to the transport. In all exposure groups, the crop was sprayed at flowering stage of the crop, during which time honeybees were actively foraging on the crop under confined conditions. The honeybees remained 5 days in the tunnels following application.

The influence of the test substances Deltamethrin EC 15 was evaluated by comparing the bees in the test cage to the control bees treated with water and those treated with the toxic standard.

The following endpoints were assessed:

- Mortality at the edge of the treated area and in the bee traps at the entrance of the colonies before as well as after the application in the control, in the treatment and reference item groups, respectively.
- Flight Intensity (number of bees that are both foraging on flowering Phacelia and flying immediately over the crop on 1 square meter) at the day the bee colonies set up into the tents and before as well as after the application in the control, in the treatment and reference item groups, respectively.
- The condition of the colonies and the development of the bee brood were checked 2 days before application and 5 days after application. In order to record effects of the test substance, the following parameters were assessed:
  - Strength of the colony (number of combs covered with bees)
  - Presence of a healthy queen (presence of eggs, presence of queen cells)
  - Estimate of the pollen storage area and area with nectar
  - Estimate of the area containing eggs, larvae and capped cells

  The amount of eggs, larvae and capped brood was given in percent of total brood population for each type of brood.
- Behaviour of the bees on the crop and around the hive

Dates of Work: 29th June 2000 to 11ths July 2000

Findings:

Honey Bee Mortality

During the pre-application period an average of 38.5 dead bees/colony/day was found in the Deltamethrin EC 15 treatment group. In the control group the average daily pre-application level of mortality was 57.9 dead bees/colony/day compared to 266 dead bees/colony/day in the toxic standard group.

An increase of the mortality was observed in the Deltamethrin EC 15 treatment group on day 0 after application (0DAT) (133.7 dead bees/colony) which was determined to be not significantly different to the control (74.3 dead bees/colony). A drastically increase of mortality was observed after application of the toxic standard with an average of 533.7 dead bees/colony on the day of application, which was significantly different to the control.

The value for $Q_{(T/Caa)}$ (mortality on the day of application divided by the average pre-application mortality) was 3.5 in the Deltamethrin EC 15 treatment group compared to 1.3 in the control group and 20.1 in the toxic standard treatment.

On evaluation days 1DAA to 5DAA the average mortality values ranged from 11.0 to 24.7 dead bees/colony/day in the Deltamethrin EC 15 treatment group. The average post-application mortality in this treatment group was 37.0 dead bees/colony/day. The mean post-application mortality in the control group was 38.5 dead bees/colony/day and 123 dead bees/colony/day in the toxic standard treatment.

When comparing the average pre-application mortality and the average post application mortality utilising $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality) no increase of mortality occurred in the test substance treatment group Deltamethrin EC 15. An obvious increase was observed in the toxic standard group. The values for $Q_{M(average)}$ were 1.0 for the test substance treatment, 0.7 for the control group and 4.6 for the toxic standard treatment.

A summary of the daily mortality and flight intensity results are shown in the following tables.

Table 2: Individual results of the evaluations of mortality (numbers of dead bees) in the Deltamethrin EC 15 at 7.5 g a.s./ha (AE F032640 00 EC02 A804) group

Date	Day	Colony 1		Colony 2		Colony 3		∑/ Colony and day
		BT	E	BT	E	BT	E	
03JULOO	3DBT	2	26	1	69	1	62	53.7
04JULOO	2DBT	0	26	2	34	1	44	35.7
05JULOO	1DBT	0	19	3	17	4	39	27.3
06JULOO	0DBT	3 / IP	19	3	16	5	65	37.3
Ø pre-application		1.5	22.5	2.3	34.0	2.8	52.5	38.5
STD		1.9	4.0	1.0	24.8	2.1	12.9	11.0
06JULOO	0DAT	4	92	8	75	25	197	133.7
07JULOO	1DAT	0	19	1	26	0	29	21.7
08JULOO	2DAT	2 / IP	15	1	25	2 / IP	27	24.7
09JULOO	3DAT	1	4	1	16	0	11	11.0
10JULOO	4DAT	I / IP	9	IP	16	1	10	13.0
11JULOO	5DAT	I / IP	18	2	12	2	18	18.0
Ø post-application		2.0	24.5	2.3	28.3	5.2	48.7	37.0
STD		1.4	33.4	2.8	23.5	9.8	73.1	47.6
Q_M		1.3	1.1	1.0	0.8	1.9	0.9	n.d.
$Q_{M(Oaa)}$		3.5						n.d.
$Q_{M(average)}$		1.0						n.d.

DBT = Days after treatment
DAT = Days after treatment
BT = Bee traps
E = Edge of the treated Phacelia area
P = Pupae
STD = Standard deviation (calculation by Quattro Pro)
Oba = Mortality on the day of treatment before application
Oaa = Mortality on the day of treatment after application
Q_M = Post-application mortality / pre-application mortality
$Q_{M(Oaa)}$ = Ø Mortality on the day of application Oaa / Ø pre-application mortality
$Q_{M(average)}$ = Ø post-application mortality / Ø pre-application mortality

Table 3: Individual results of the evaluations of mortality (numbers of dead bees) in the control group

Date	Day	Colony 1		Colony 2		Colony 3		Ø / Colony and day
		BT	E	BT	E	BT	E	
03JULOO	3DBT	0	34	0	99	2	41	58.7
04JULOO	2DBT	2	51	0	115	0	34	67.4
05JULOO	1DBT	4/ 2P	22	2/2P	66	3	29	43.3
06JULOO	0DBT	1/ 3P	35	4 / 1P	119	1/ 1P	21	62.0
Ø pre-application		3.0	35.5	2.3	99.8	1.8	31.3	57.9
STD		2.6	11.9	2.6	24.1	1.3	8.4	10.3
06JULOO	0DAT	11	53	3	106	0	50	74.3
07JULOO	1DAT	0	13	0	48	0	18	26.3
08JULOO	2DAT	6	16	4/ 1P	68	1/ 1P	25	40.7
09JULOO	3DAT	3P	10	3/ 1P	34	0	18	23.0
10JULOO	4DAT	0	21	1P	35	1	25	27.7
11JULOO	5DAT	IP	31	2/ 5P	46	3	28	38.7
Ø post-application		3.5	24.0	3.3	56.2	1.0	27.3	38.5
STD		4.3	16.0	2.6	27.3	1.3	11.8	18.9
Q_M		1.2	0.7	1.4	0.6	0.6	0.9	n.d.
$Q_{M(Oaa)}$		1.3						n.d.
$Q_{M(average)}$		0.7						n.d.

DBT = Days after treatment
DAT = Days after treatment
BT = Bee traps
E = Edge of the treated Phacelia area
P = Pupae
STD = Standard deviation (calculation by Quattro Pro)
Oba = Mortality on the day of treatment before application
Oaa = Mortality on the day of treatment after application
Q_M = Post-application mortality / pre-application mortality
$Q_{M(Oaa)}$ = Ø Mortality on the day of application Oaa / Ø pre-application mortality
$Q_{M(average)}$ = Ø post-application mortality / Ø pre-application mortality

Table 4: Individual results of the evaluations of mortality (numbers of dead bees) in the toxic standard group

Date	Day	Colony 1		Colony 2		Colony 3		Ø / Colony and day
		BT	E	BT	E	BT	E	
03JULOO	3DBT	12P	11	1	7	3	25	19.7
04JULOO	2DBT	1/ 21P	10	2	6	2P	24	22.0
05JULOO	1DBT	3/ 23P	22	5	11	11P	40	38.3
06JULOO	0DBT	1/ 1P	15	1/ 7P	16	7P	31	26.4
Ø pre-application		15.5	14.5	4.0	10.0	5.8	30.3	26.6
STD		10.8	5.4	3.2	4.5	4.1	7.1	8.3
06JULOO	0DAT	202	444	59	149	298	449	533.7 A
07JULOO	1DAT	14	80	7	9	39	57	68.7
08JULOO	2DAT	30	57	27	28	70	90	100.6
09JULOO	3DAT	2	15	1	2	1/ 8P	6	11.7
10JULOO	4DAT	1/ 1P	6	0	10	1P	5	8.0
11JULOO	5DAT	2/4P	4	2/ 3P	21	0	9	15.0
Ø post-application		42.7	101.0	16.5	36.50	69.5	104.3	123
STD		78.8	170.8	23.0	55.9	115.2	172.6	204.6
Q_M		2.8	7.0	4.1	3.7	12.0	3.4	n.d.
$Q_{M(Oaa)}$		20.1						n.d.
$Q_{M(average)}$		4.6						n.d.

DBT = Days after treatment
DAT = Days after treatment
BT = Bee traps
E = Edge of the treated Phacelia area
P = Pupae
STD = Standard deviation (calculation by Quattro Pro)
Oba = Mortality on the day of treatment before application
Oaa = Mortality on the day of treatment after application
Q_M = Post-application mortality / pre-application mortality
$Q_{M(Oaa)}$ = Ø Mortality on the day of application Oaa / Ø pre-application mortality
$Q_{M(average)}$ = Ø post-application mortality / Ø pre-application mortality
A significantly higher compared to the control (Dunnett-Test; a = 0.05)

Honey Bee Flight Intensity

Due to bad weather conditions (rain) almost no flight intensity was observed on DAA-2, 2 and 3.

The average flight intensity during the pre-application period was similar in all treatment groups. In the Deltamethrin EC 15 treatment group an average of 8.3 bees/m² was observed visiting the flowering Phacelia during the pre-application period compared to 7.4 bees/m² in the control group and 6.0 bees/m² in the toxic standard treatment.

Shortly before application an average of 16.7 bees/m² was observed in the Deltamethrin EC 15 treatment group foraging on the flowering Phacelia. The mean flight intensity of 9.2 bees/m² in the test substance treatment group and 4.8 bees/m² of the toxic standard treatment on day DAA Oaa remained significantly below the level of the control group (23.2 bees/m²). Furthermore the flight intensity in the Deltamethrin EC 15 treatment group and in the toxic standard treatment on evaluation day DAA 1 was significantly lower compared to the control. Compared with the pre-application period the average daily post-application level of flight intensity was lower in the test substance treatment group Deltamethrin EC 15 and in the toxic standard treatment but higher in the control group.

The average post-application level of flight intensity was 6.1 bees/m² in the Deltamethrin EC 15

treatment group, 2.9 bees/m^2 in the toxic standard group and 10.3 in the control.

Table 5: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the Deltamethrin EC 15 at 7.5 g a.s/ha group

Date	DAA	Ø Number of bees/m^2			Ø Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
03JUL00	-3	23	7	11	13.7
04JUL00	-2	0	0	0	0.0
05JUL00	-1	2	4	4	3.0
06JUL00	0ba	18	16	16	16.7
Ø pre-application.		10.8	6.5	7.8	8.3
STD		11.5	7.0	7.1	8.2
06JUL00	0aa	9.7	7.8	16.2	9.2[1]
07JUL00	1	26	16	19.7	18.6
08JUL00	2	0	0	0	0.0
09JUL00	3	0	0	0	0.0
10JUL00	4	1	1	1	1.0
11JUL00	5	6	12	6	8.0
Ø post-application.		6.4	6.1	6.2	6.1
STD		7.8	6.9	7.8	7.3

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application
[1] significantly reduced compared to the control (Dunnett-Test; a = 0.05

Table 6: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the control group

Date	DAA	∅ Number of bees/m^2			∅ Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
03JUL00	-3	9	6	2	5.7
04JUL00	-2	0	0	0	0.0
05JUL00	-1	11	3	5	6.3
06JUL00	0ba	17	18	18	17.7
∅ pre-application.		9.3	6.8	6.3	7.4
STD		7.0	7.9	8.1	7.1
06JUL00	0aa	24.4	24	22.2	23.2
07JUL00	1	33.7	26.7	22	27.5
08JUL00	2	0	0	0	0.0
09JUL00	3	0	0	0	0.0
10JUL00	4	2	1	1	1.3
11JUL00	5	9	7	13	9.7
∅ post-application.		11.5	9.6	9.7	10.3
STD		14.3	12.1	10.8	12.3

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application

Table 7: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the toxic standard group

Date	DAA	∅ Number of bees/m^2			∅ Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
03JUL00	-3	2	10	4	5.3
04JUL00	-2	0	0	0	0.0
05JUL00	-1	3	0	1	1.3
06JUL00	0ba	18	10	28	17.3
∅ pre-application.		5.8	6.5	5.8	6.0
STD		8.3	7.2	8.3	7.4
06JUL00	0aa	5.3	3	6.2	4.8
07JUL00	1	3.3	2.3	4	3.4[1]
08JUL00	2	0	0	0	0.0
09JUL00	3	0	0	0	0.0
10JUL00	4	0	0	1	0.3
11JUL00	5	9	6	11	8.7
∅ post-application.		2.9	1.9	3.8	2.9
STD		3.7	2.4	4.4	3.5

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application

[1] significantly reduced compared to the control (Dunnett-Test; a = 0.05)

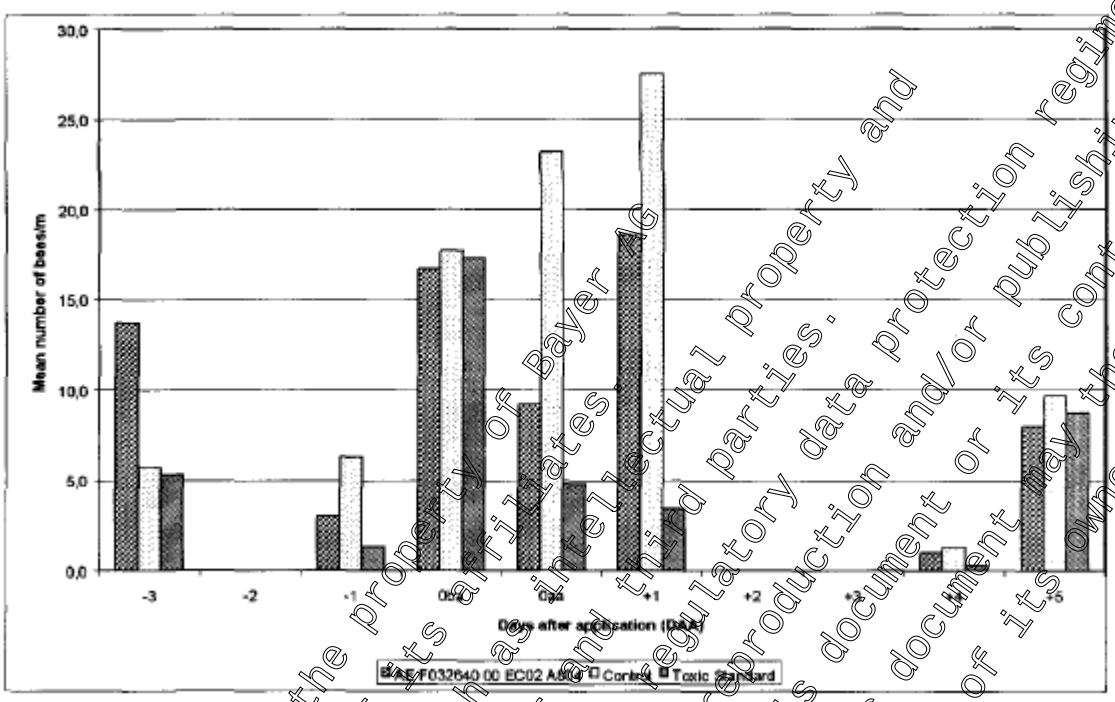


Figure 1: Average flight intensity in the test substance treatment group Deltamethrin EC 15 at 7.5 g a.s./ha (AE F032640 00 EC02 A804), the control and the toxic standard group prior to and after application

Oba = evaluation on the day of treatment shortly before application
Oaa = evaluation on the day of treatment after application

Development of honey bee brood

During the observation period changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred in almost every colony of the test substance groups, control and toxic standard group.

Compared to the brood assessment at the beginning of the test, no decrease of the amount of the different brood stages and the strength of the colonies could be observed in the hives of the test substance treatment at the assessment five days after application. In the two queen-right colonies of the control group less egg and larval stages were observed at the second brood assessment. In the toxic standard group a clear decrease of larval stages was observed during the test period. The number of combs covered with brood was lower at the assessment five days after application.

The continued presence of eggs showed that the queens survived in all colonies except in colony No. 3 of the control group.

Table 8: Brood development of the Deltamethrin EC 15 at 7.5 g a.s/ha (AE F032640 00 EC02 A804) group

	Colony 1	Colony 2	Colony 3
Prior to application: 04JUL00			
Strength (No. of combs covered with bees)	3.0	3.0	3.5
No. of combs covered with brood	2	3	3
Average amount of egg stage in %	22.5	15.0	11.7
Average amount of larval stage in %	7.5	23.3	1.7
Average amount of capped stage in %	30.0	20.0	33.3
After application: 11JUL00			
Strength (No. of combs covered with bees)	3.0	3.5	3.5
No. of combs covered with brood	2	3	2
Average amount of egg stage in %	15.0	8.3	20.0
Average amount of larval stage in %	27.5	20.0	17.5
Average amount of capped stage in %	30.0	20.0	22.5

Table 9: Brood development of the control group

	Colony 1	Colony 2	Colony 3
Prior to application: 04JUL00			
Strength (No. of combs covered with bees)	3.5	3.0	3.0
No. of combs covered with brood	2	3	2
Average amount of egg stage in %	30.0	26.0	0.0
Average amount of larval stage in %	7.5	11.7	0.0
Average amount of capped stage in %	32.5	18.3	27.5
After application: 11JUL00			
Strength (No. of combs covered with bees)	3.0	3.0	2.5
No. of combs covered with brood	2	2	2
Average amount of egg stage in %	5.0	12.5	0.0
Average amount of larval stage in %	5.0	10.0	0.0
Average amount of capped stage in %	22.5	22.5	10.0

Behaviour of the Bees

Directly after application of Deltamethrin EC 15 the bees were observed rising up of the flowering Phacelia, and the bees were observed landing on the flowers but immediately afterwards flying back to the hives. Due to this observation a repellent effect of the test substance during a short time after application can be assumed. Approximately one hour after application affected and cramping bees were found on the linen. Bees which returned to the hives were observed cleaning their wings.

Conclusion:

The side effects of the test substance Deltamethrin EC 15 (AE F032640 00 EC02 A804) was tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the EPPO guideline No. 170: Guideline on test methods for evaluation the sideeffects of plant protection products on honey bees (EPPO, 1992).

The test substance was applied at an application rate of 7.5 g a.i./ha in 300 L water/ha.

Mortality: The application of the test substance Deltamethrin EC 15 resulted in an increase of mortality restricted to the day of application (DAA Oaa). An average of 133.7 dead bees/colony was found in the test substance treatment which was determined to be not significantly different to the control (74.3 dead bees/colony). A drastically increase of mortality was observed after application of the toxic standard with an average of 533.7 dead bees/colony. The effect of the toxic standard demonstrated the sensitivity of the method in detecting the toxic effects of a pesticide.

When comparing the average pre-application mortality and the average post-application mortality utilising $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality) no increase of mortality occurred in the test substance treatment group Deltamethrin EC 15. The values for $Q_{M(average)}$ were 1.0 for the test substance treatment, 0.7 for the control group and 4.6 for the toxic standard treatment.

Flight intensity: In the Deltamethrin EC 15 group an obvious repellent effect occurred directly after application assumed by the behaviour of the bees and confirmed due to the flight intensity (9.2 bees/m2) on this day which remained significantly below the level of the control group (23.2 bees/m2). The significantly reduced flight intensity in the Deltamethrin EC 15 4 treatment group and in the toxic standard treatment lasted until evaluation day 1DAT.

Compared with the pre-application period the average daily post-application level of flight intensity was lower in the test substance treatment group Deltamethrin EC 15 and in the toxic standard treatment but higher in the control group.

Brood development: Regarding the colonies strength and the bee brood development no abnormal differences attributable to the influence of the test substance were observed between the test substance groups and the control.

*****

Report:	**KCP 10.3.1.5/07, [redacted], 2001**
Title:	Assessment of effects on honeybees of AE F032640 00 EC03 A1 and AE F032640 00 EW01 B1 - Trial under Insectproof tunnels on *Phacelia* crop.
Document No:	M-205048-01-1 (Rep. No.: 36-2001)
Guidelines:	CEB 129
GLP:	yes

~~**Material and Methods:**~~

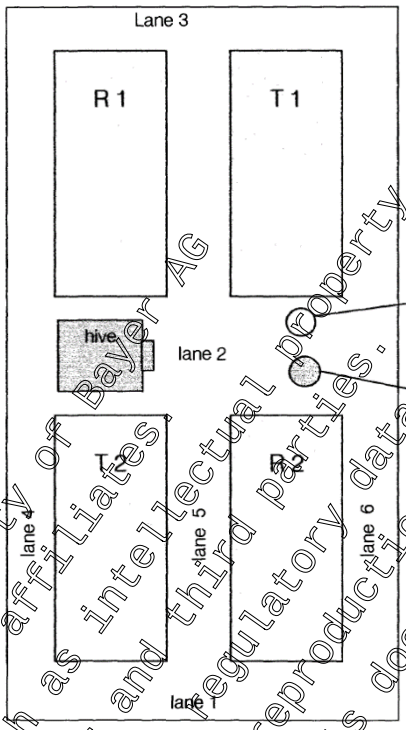
~~Honey bee colonies (ca 16,000 to 19,000 bees per hive, colonies of comparable development, brood, and food store status, queens of homogeneous maternal origin) were confined in tunnels on *Phacelia* fields with additional pollen sources provided. Five days after introduction of the bees into the tunnels, application was performed. The test substance Deltamethrin EW 15 was applied at rates of 0.333 L/ha and 0.500 L/ha, the toxic standard was Zolone Flo (500 g/L phosalone) at a rate of 1.2 L/ha. Furthermore,~~

~~a water-treated control was set up. Treatment was carried out during flight activity of the bees. Endpoints observed were foraging activity, behavior, mortality, and colony development.~~

~~**Findings:**~~
~~Behavior of the bees was only slightly affected by the test item as well as by the standard. Foraging activity was influenced by the test substance only for a short time. Mortality increased shortly after test item appliaction at both rates but dropped down to levels comparable to control soon after application. Colony development was not affected by the treatment.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EW 15 (AE F032640 00 EW01 B1) content of a.s.: deltamethrin: (analysed): 15.1 g/L (15.0 g/L nominal) Deltamethrin EC 25 (AE F032640 00 EC03 A1) content of a.s. (analysed): 24.9 g/L (25 g/L nominal)
Batch number:	Deltamethrin EW 15: OP200456 Deltamethrin EC 25: OP200859
Reference item:	Zolone Flo (active ingredient: phosalone, 500 g a.s./L nominal, analysed content: 499 g a.s./L)
Test organism:	Honey bees (*Apis mellifera*) The used hives were of the DADANT 10 frames model, with one queen and approximately 16000 to 19000 bees per hive at test start. The colonies were as homogeneous as possible. The corresponding queens (Italian breed) were one year old and originated from one breeding line in order to guarantee uniform bee material in all treatment groups. Honeybees spread over 7 to 8 frames (of which approximately 4 to 6 frames of brood)
Source:	Not stated in the report
Crop:	*Phacelia tanacetifolia* (bee attractive crop) at flowering stage
Test unit:	Each tunnel covered an area of 140 m^2 with a roof height of approximately 3 metres. The tunnel tent frames were covered by a polyethylene mesh net (1.2 mm × 1.2 mm). Inside the tunnels the Phacelia crop was split into four plots with 16 m^2 (8 m × 2 m) each. A beehive, a watering place and feeders with pollen were placed in each of the tunnels and supplied daily. Tunnels were spaced out 3 metres from another. A design of a tunnel is presented below.



R: sheltered area
T: treated area

Application rates:	Control: water Deltamethrin EC 25: 0.200 L/ha (5.0 g a.s./ha) during foraging activity Deltamethrin EW 15: 0.333 L/ha (5.0 g a.s./ha) during foraging activity Deltamethrin EW 15: 0.500 L/ha (7.5 g a.s./ha) during foraging activity Reference rate (Zolone Flo): 1.20 L/ha (600 g a.s./ha) One product was tested in each tunnel. The spray volume was 300 to 315 L/ha in all treatment groups.
Data analysis:	The data of mortality, foraging, condition of the colonies and bee behaviour of the test item group and the reference item group were compared to the control.
Data sampling:	Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.
Deviations from the study plan:	Study item was coded as Deltamethrin EW 15 but this code indicates AE F032640 00EW01 B1

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperatures favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table.

Table 1: Environmental conditions during the experimental period

Parameter	Experimental period										
	25/8/01	26/8/01	27/8/01	28/8/01	29/8/01	30/8/01	31/8/01	1/9/01	2/9/01	3/9/01	4/9/01
Rainfall [mm]	0	0	0	0	0	2	0	0	0	0	4
Mmin T [°C]	15	17	19	13	15	15	10	9	6	10	9
Max T [°C]	35	31	26	26	26	19	20	22	24	18	19

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 2: Phacelia crop data, 2001 campaign

Date	Operation	Characteristics
April	Soil preparation	Herbicide application and harrowing: seedbed preparation and weed destruction
15/06/01	Plot sowing and rolling	*Phacelia tanacetifolia* variety: Balo, at 5 kg/ha (Batch D/BN 228-0-92-17)
August	Destruction	Crushing the crop on experimental plots

This study included five exposure groups with one replicate (tunnels) each: one tap-water treated control group, three test-item groups (1 × Deltamethrin EC 25 and 2 × Deltamethrin EW 15) and one reference item group. In all exposure groups, the crop was sprayed 5 days after set-up of the hives in the tunnels at full-flowering, during which time honeybees were actively foraging on the crop under confined conditions. The honeybees remained 11 days in the tunnels.

Mortality in each tunnel was recorded on a daily basis for all areas covered with plastic film, from 4 days before treatment (4DBT) to six days after treatment (6DAT). Moreover, the day on which product application was carried out (day 0) additional counts were done at the end of the day (0DAT) in order to establish possible bucal intoxication of foraging bees. The total mortality rate recorded in a tunnel for a given day results from adding up mortality rates observed in each of the six plastic rows in the tunnel.

Foraging was observed from 2DBT to 2DAT, on all treated and sheltered (untreated) areas. It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts were shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall, etc.) This parameter was also taken into account for an additional count on D 0, during the hour following product application.

Two colony assessments were carried out in the beginning and at the end of experimentation, allowing to evaluate colony development taking into account parameters like the adult bee population, the quantity and quality of the brood (different stages observed), amount of reserves and potential construction of new frames on offered wax sheets. These visits were carried out in the tunnels at dates which were as close as possible to the first and last day of confinement. However, for practical or climatic conditions, they necessarily took place within 48 hours before or after introduction of the hives in the tunnels on the one hand, and when the hives were taken out on the other hand.

Assessments of bee behaviour were carried out when products were applied and during the thirty minutes following product application, In general, this observation phase continued all over the day, between counts. Bees were especially observed for reactions and behaviour like intense flying, bee clusters on the net or at the entrance of the hive, aggressiveness, beginning of an intoxication etc. in each of the tunnels.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment groups to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Number of foraging bees/m^2 per day on all the areas (T1, T2 and R1, R2) before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the test item groups and the reference item group, respectively
- Colony Assessment in the beginning and at the end of experimentation

Dates of Work: 25th August to 04th September 2001

Findings:

Honey Bee Mortality:

A summary of the daily mortality and total mortality results are shown in the following table.

Table 3: Daily mortality data

Treatment	4DBT – 25th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	33	5	76	14	2	16	146
Deltamethrin EC 25 (5.0 g a.s./ha)	27	11	153	52	6	8	257
Deltamethrin EW 15 (5.0 g a.s./ha)	47	43	93	32	3	15	233
Deltamethrin EW 15 (7.5 g a.s./ha)	53	17	67	7	4	11	159
Zolone Flo	94	44	156	26	10	33	363
	3DBT – 26th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	211	24	85	98	14	95	527
Deltamethrin EC 25 (5.0 g a.s./ha)	61	11	101	67	5	32	277
Deltamethrin EW 15 (5.0 g a.s./ha)	131	32	83	114	12	33	405
Deltamethrin EW 15 (7.5 g a.s./ha)	92	25	112	91	14	38	372
Zolone Flo	107	33	122	69	2	88	421
	2DBT – 27th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	154	9	25	21	6	32	247

Deltamethrin EC 25 (5.0 g a.s./ha)	144	24	67	80	8	30	353
Deltamethrin EW 15 (5.0 g a.s./ha)	208	23	89	40	16	40	416
Deltamethrin EW 15 (7.5 g a.s./ha)	252	17	75	51	5	60	460
Zolone Flo	350	27	88	70	20	115	670
	1DBT – 28th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	55	5	111	55	6	8	240
Deltamethrin EC 25 (5.0 g a.s./ha)	47	17	102	169	4	10	349
Deltamethrin EW 15 (5.0 g a.s./ha)	116	14	251	169	26	38	605
Deltamethrin EW 15 (7.5 g a.s./ha)	97	19	173	80	9	12	390
Zolone Flo	202	13	220	164	21	17	637
	0DBT – 29th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	23	13	197	90	6	11	340
Deltamethrin EC 25 (5.0 g a.s./ha)	27	12	120	192	0	6	357
Deltamethrin EW 15 (5.0 g a.s./ha)	31	15	159	110	5	20	340
Deltamethrin EW 15 (7.5 g a.s./ha)	42	16	270	82	11	18	439
Zolone Flo	63	20	355	261	9	22	730
	0DAT – 29th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	75	1	42	42	8	13	179
Deltamethrin EC 25 (5.0 g a.s./ha)	122	14	63	60	4	28	291
Deltamethrin EW 15 (5.0 g a.s./ha)	194	20	106	56	8	46	430
Deltamethrin EW 15 (7.5 g a.s./ha)	307	21	161	61	13	66	629
Zolone Flo	120	25	88	58	6	66	363
	1DAT – 30th August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	16	4	14	3	4	15	56
Deltamethrin EC 25 (5.0 g a.s./ha)	77	10	52	25	5	24	193
Deltamethrin EW 15 (5.0 g a.s./ha)	103	15	92	30	7	48	295
Deltamethrin EW 15 (7.5 g a.s./ha)	139	18	78	30	14	53	332
Zolone Flo	29	25	31	17	5	30	137
	2DAT – 31st August 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	98	8	7	7	8	15	143
Deltamethrin EC 25 (5.0 g a.s./ha)	168	19	26	14	9	30	266
Deltamethrin EW 15 (5.0 g a.s./ha)	288	18	41	18	22	40	427

Deltamethrin EW 15 (7.5 g a.s./ha)	255	10	12	9	11	28	325
Zolone Flo	222	15	11	8	5	24	285
	3DAT – 1st September 2001						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	144	2	6	10	7	12	181
Deltamethrin EC 25 (5.0 g a.s./ha)	118	8	5	12	4	34	181
Deltamethrin EW 15 (5.0 g a.s./ha)	195	8	17	8	10	27	265
Deltamethrin EW 15 (7.5 g a.s./ha)	170	2	3	9	5	19	208
Zolone Flo	201	6	6	12	6	33	264
20 July	**4DAT – 2nd September 2001**						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	172	16	50	49	10	16	313
Deltamethrin EC 25 (5.0 g a.s./ha)	95	17	48	59	7	21	247
Deltamethrin EW 15 (5.0 g a.s./ha)	206	11	63	38	6	28	352
Deltamethrin EW 15 (7.5 g a.s./ha)	143	7	39	23	5	9	226
Zolone Flo	227	7	98	60	6	46	442
21 July	**5DAT – 3rd September 2001**						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Water control	44	9	21	43	5	26	148
Deltamethrin EC 25 (5.0 g a.s./ha)	55	11	21	56	7	36	186
Deltamethrin EW 15 (5.0 g a.s./ha)	92	17	32	31	5	61	238
Deltamethrin EW 15 (7.5 g a.s./ha)	50	13	22	23	2	14	124
Zolone Flo	107	7	29	32	3	47	225
	6DAT – 4th September 2001						
Water control	38	8	25	7	7	5	90
Deltamethrin EC 25 (5.0 g a.s./ha)	15	6	6	15	4	6	52
Deltamethrin EW 15 (5.0 g a.s./ha)	23	19	22	13	8	9	94
Deltamethrin EW 15 (7.5 g a.s./ha)	21	6	8	7	7	3	52
Zolone Flo	39	9	4	5	5	3	65

DBT: days before treatment
DAT: days after treatment

The total mortality is displayed in the figure below.

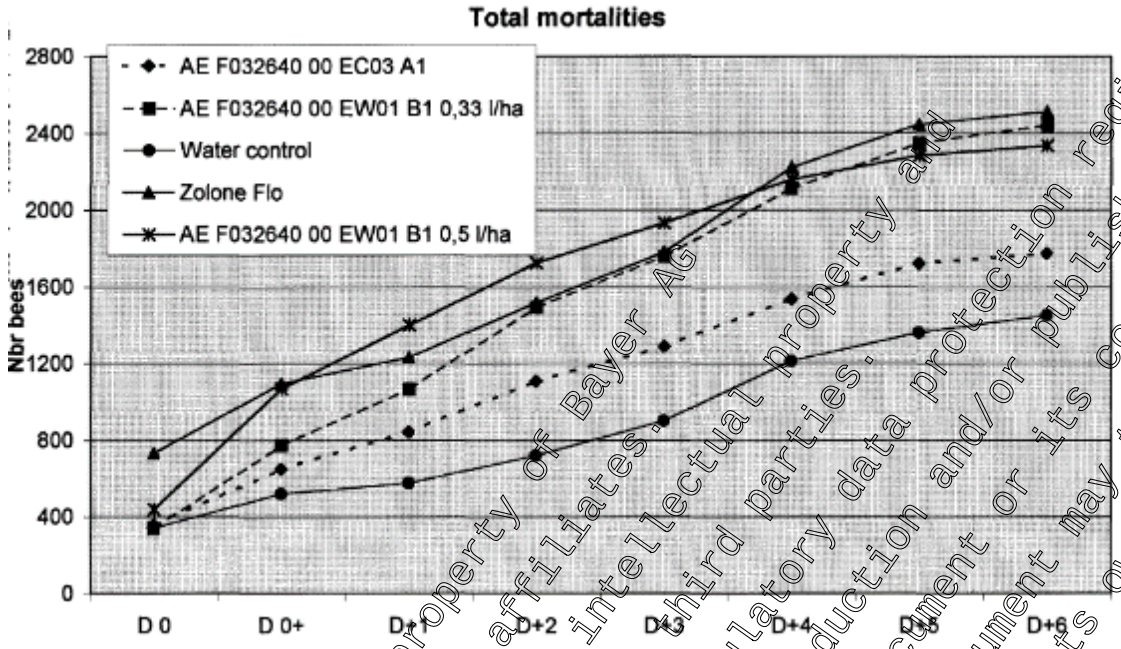


Figure 1: Total mortalities during study period for Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 5.0 g a.s./ha, Deltamethrin EW 15 (AEF032640 00 EW01 B1 - 0.33 l/ha) at 5.0 g a.s./ha, for the water control group, the reference group (Zolone Flo) and Deltamethrin EW 15 G (AEF032640 00 EW01 B1 - 0.5 l/ha) at 7.5 g a.s./ha
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+6: 1 to 6 days after treatment

One day after treatment, in both control and reference tunnels mortality decreased, as water treatment or Zolone application did not disturb bees. On the contrary mortalities increasd in all study items tunnels. The difference is that AE F032640 00 EC03 A1 formulation induced a very small increase in mortality when Deltamethrin EW 15 at two different rates provided little peaks of mortality. A main peak was observed with the higher dose used (0.5 L/ha).

Then during the second part of this trial, evolution of mortalities were similar within all tunnels. In a way all mortality evolutions were comparable along this two weeks trial, as there is no toxic reference. The highest peak of mortality was under 1 000 individuals, when a toxic was supposed to provide much more dead bees. So all recorded data had to be considered as inducing moderate mortalities, the more so as the effects were limited to the following treatment day (1DAT) only.

On the product application day, between mortality records made in the morning (0DBT) and those made in the evening (0DAT), increasing curves gave information about knockdown effects. Only the highest dose of Deltamethrin EW 15 (7.5 g a.s./ha) provided an early high mortality during the afternoon. That was still obvious on the following morning day.

Of course the water control induced the lowest increase in daily mortality. From 1DAT to 6DAT all evolutions were similar, that confirmed the limited in time effects of both Deltamethrin EC 25 and Deltamethrin EW 15 formulations.

At the end of this experimental phase, total mortalities were split up in two groups: Deltamethrin EC 25 effects were comparable to the control, whereas the two doserates of Deltamethrin EW 15 and the reference Zolone Flo were linked together.

Foraging activity

The mean number of forager bees/m² inside the tunnels for all treatments is shown in the following tables.

Table 4: Foraging data: Water control

Water control		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 27th 01	14H00	116	43	90	138	65	120	117	40	194	171	12,1	10,7
D-2	15H00	115	51	98	165	84	126	136	42	215	194	13,4	12,1
	16H30	115	65	107	158	76	140	137	52	223	203	13,9	12,7
											189		11,8
August 28th 01	12H00	74	41	74	141	69	105	104	39	165	159	10,3	9,9
D-1	14H30	137	78	93	164	93	138	158	77	236	233	14,8	14,6
	16H00	152	72	125	183	115	166	150	67	266	249	16,6	15,6
											214		13,3
August 29th 01	12H00	103	44	90	119	75	98	112	45	178	165	11,1	10,3
	13H30	151	87	130	186	123	150	170	74	277	259	17,3	16,2
D 0											212		13,2
	15H00	120	92	109	179	93	150	150	66	250	233	15,6	14,5
	15H45	103	61	77	133	72	109	104	59	187	172	11,7	10,8
	16H30	80	48	89	114	79	100	90	43	166	156	10,3	9,8
											187		11,7
August 30th 01	16H00	31	29	31	33	35	43	48	18	non significant data. non taken into account			
D+1	18H30	/	/	/	/	/	/	/	/				
August 31st 01	13H30	66	108	98	102	97	81	72	98	187	174	11,7	10,9
D+2	15H00	71	125	84	72	145	63	59	123	176	195	11,0	12,2
	16H30	73	48	61	81	60	78	74	49	132	127	8,2	7,9
											165		10,3
September 1st 01	11H00	82	77	87	91	83	93	114	89	169	190	10,5	11,8
D+3	12H30	121	80	109	121	90	117	143	99	216	225	13,5	14,0
											207		12,9

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-2/ D-1: 2/1 days before treatment
D0: day of treatment
D+1 to D+3: 1 to 3 days after treatment

Table 5: Foraging data: Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 5.0 g a.s./ha

AE F032640 00 EC03 A1		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 27th 01	14H00	66	85	61	83	54	70	94	86	148	152	9,2	9,5
D-2	15H00	89	79	62	109	57	83	78	90	170	154	10,6	9,6
	16H30	89	95	84	93	74	89	97	108	181	184	11,3	11,5
											163		10,2
August 28th 01	12H00	41	40	57	52	47	47	62	58	95	107	5,9	6,7
D-1	14H30	89	103	70	96	58	80	108	89	179	168	11,2	10,5
	16H00	98	97	82	113	81	91	107	114	195	197	12,2	12,3
											157		9,8
August 29th 01	12H00	38	46	48	66	41	35	70	72	99	109	6,2	6,8
	13H30	92	101	98	95	88	93	114	131	193	209	12,1	13,0
D 0											159		9,9
	15H00	57	74	59	84	36	54	34	40	127	82	7,9	5,1
	15H45	37	54	46	57	31	37	31	47	97	73	6,1	4,6
	16H30	34	65	50	64	43	38	32	48	107	81	6,7	5,0
											79		4,9
August 30th 01	16H00	30	34	34	32	33	40	47	45	non significant data, non taken into account			
D+1	18H30	25		21	/	19	/	29	/				
	12H00	27	33	14	22	20	25	31	23	non significant data			
August 31st 01	13H30	44	40	41	68	50	55	56	44	97	103	6,0	6,4
D+2	15H00	87	50	62	87	110	66	64	71	148	166	9,3	10,3
	16H30	44	52	36	44	44	57	52	63	88	108	5,5	6,8
											125		7,8
September 1st 01	11H00	57	55	59	78	58	83	63	73	125	139	7,8	8,7
D+3	12H30	73	92	108	104	95	93	124	116	189	214	11,8	13,4
											176		11,0

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16, same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-2 / D-1: 2/ 1 days before treatment
D0: day of treatment
D+1 to D+3: 1 to 3 days after treatment

Table 6: Foraging data: Deltamethrin EW 15 (AE F032640 00 EW01 B1) at 5.0 g a.s./ha

AE F032640 00 EW01 B1 0,33 l/ha		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 27th 01	14H00	120	93	103	157	101	135	159	119	237	257	14,8	16,1
D-2	15H00	139	105	110	147	121	145	182	124	251	286	15,7	17,9
	16H30	154	118	143	180	126	147	183	150	298	303	18,6	18,9
											282		17,6
August 28th 01	12H00	110	70	88	127	90	116	156	94	198	228	12,3	14,3
D-1	14H30	161	114	117	168	119	156	198	138	280	306	17,5	19,1
	16H00	167	147	146	187	136	159	213	163	324	336	20,2	21,0
											290		18,1
August 29th 01	12H00	104	87	109	108	93	94	114	108	204	205	12,8	12,8
	13H30	198	166	143	198	162	165	217	184	353	364	22,0	22,8
D 0											284		17,8
	15H00	87	100	91	108	58	83	71	42	193	127	12,1	7,9
	15H45	76	64	59	84	46	46	60	47	142	100	8,8	6,2
	16H30	64	82	81	71	54	48	60	58	148	110	9,3	6,9
											112		7,0
August 30th 01	16H00	63	88	44	40	57	49	79	47	non significant data. non taken into account			
D+1	18H30	33	/	26	/	28	/	37	/				
	12H00	48	30	11	9	11	14	25	23	non significant data			
August 31st 01	13H30	102	96	118	78	89	68	147	102	197	203	12,3	12,7
D+2	15H00	136	118	101	90	128	97	138	140	223	252	13,9	15,7
	16H30	91	68	66	83	74	67	79	78	154	149	9,6	9,3
											201		12,6
September 1st 01	11H00	96	86	88	103	91	97	118	105	187	206	11,7	12,8
D+3	12H30	139	89	93	120	122	131	161	111	221	263	13,8	16,4
											234		14,6

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-2, D-1: 2/ 1 days before treatment
D0: day of treatment
D+1 to D+3: 1 to 3 days after treatment

Table 7: Foraging data: Deltamethrin EW 15 (AE F032640 00 EW01 B1) at 7.5 g a.s./ha

AE F032640 00 EW01 B1 0,5 l/ha		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 27th 01	14H00	79	86	123	120	118	161	90	107	204	238	12,8	14,9
D-2	15H00	83	94	123	114	111	168	102	87	207	234	12,9	14,6
	16H30	94	92	95	121	140	193	83	94	201	255	12,6	15,9
											242		15,1
August 28th 01	12H00	72	66	94	93	101	138	71	67	163	189	10,2	11,8
D-1	14H30	118	111	103	117	138	175	99	125	225	269	14,0	16,8
	16H00	115	136	114	103	152	181	108	134	234	288	14,6	18,0
											248		15,5
August 29th 01	12H00	72	80	97	95	116	166	77	98	172	229	10,8	14,3
	13H30	117	123	121	131	162	206	116	107	246	296	15,4	18,5
D 0											262		16,4
	15H00	54	82	67	83	28	48	39	22	143	69	8,9	4,3
	15H45	37	63	62	60	44	52	23	26	111	73	6,9	4,5
	16H30	41	40	67	37	47	32	23	26	93	64	5,8	4,0
											68		4,3
August 30th 01	16H00	38	43	41	27	30	23	23	26	non significant data: non taken into account			
D+1	18H30	/	/	/	/	/	/	/	/				
August 31st 01	13H30	102	84	97	93	85	106	92	101	188	192	11,8	12,0
D+2	15H00	88	89	101	87	100	87	103	87	178	188	11,1	11,8
	16H30	30	45	53	47	42	48	40	47	88	89	5,5	5,5
											156		9,8
September 1st 01	11H00	79	97	87	90	95	100	98	109	177	201	11,0	12,6
D+3	12H30	112	115	103	109	116	124	107	118	220	233	13,7	14,5
											217		13,5

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m^2 : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m^2.
D-2/ D-1 : 2/ 1 days before treatment
D0: day of treatment
D+1 to D+3: 1 to 3 days after treatment

Table 8: Foraging data: Reference item Zolone Flo

Zolone Flo		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 27th 01	14H00	123	76	113	139	93	147	111	99	226	225	14,1	14,1
D-2	15H00	188	70	129	146	96	137	111	77	267	210	16,7	13,2
	16H30	182	84	125	146	101	124	135	83	269	222	16,8	13,8
											219		13,7
August 28th 01	12H00	122	66	74	106	73	108	106	64	184	177	11,5	11,0
D-1	14H30	170	93	94	169	91	160	146	89	263	243	16,4	15,2
	16H00	209	103	95	161	123	156	165	98	284	271	17,8	16,9
											230		14,4
August 29th 01	12H00	120	66	76	100	87	100	106	92	181	193	11,3	12,0
	13H30	197	134	129	176	132	175	159	142	318	304	19,9	19,0
D 0											248		15,5
	15H00	155	118	126	159	99	128	156	131	279	254	17,4	15,9
	15H45	141	106	94	132	112	131	89	142	237	237	14,8	14,8
	16H30	107	59	70	93	84	93	75	82	165	157	10,3	9,8
											216		13,5
August 30th 01	16H00	77	43	45	47	38	52	44	44	non significant data, non taken into account			
D+1	18H30	/	/	/	/	/	/	/	/				
August 31th 01	13H30	91	126	65	82	95	77	96	76	182	172	11,4	10,8
D+2	15H00	77	136	83	91	151	84	95	62	184	196	11,5	12,3
	16H30	76	50	30	56	41	57	43	44	106	93	6,6	5,8
											154		9,6
September 1st 01	11H00	122	84	79	81	89	78	91	88	183	173	11,4	10,8
D+3	12H30	140	93	98	101	102	115	114	107	216	219	13,5	13,7
											196		12,3

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m^2 : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m^2.
D-2/ D-1: 2/ 1 days before treatment
D0: day of treatment
D+1 to D+3: 1 to 3 days after treatment

From the beginning of this trial, following introduction of the beehives in the tunnels, bees foraged in the crop quite actively.

During the three counts that followed product applications, mean foraging trends were comparable between 2 tunnel groups. The colony in the reference tunnel seemed to be indifferent to phosalone application. On the same way in the control tunnel, water spraying did not disturb the foragers activity. Foraging activity diminished shortly after treatment in these tunnels. On the contrary this foraging activity literally droped in the Deltamethrin EW 15 formulation tunnels and diminished in Deltamethrin EC 25 too.

In the figure below foraging activity shortly before treatment is compared to foraging activity shortly after treatment in the sheltered and treated areas.

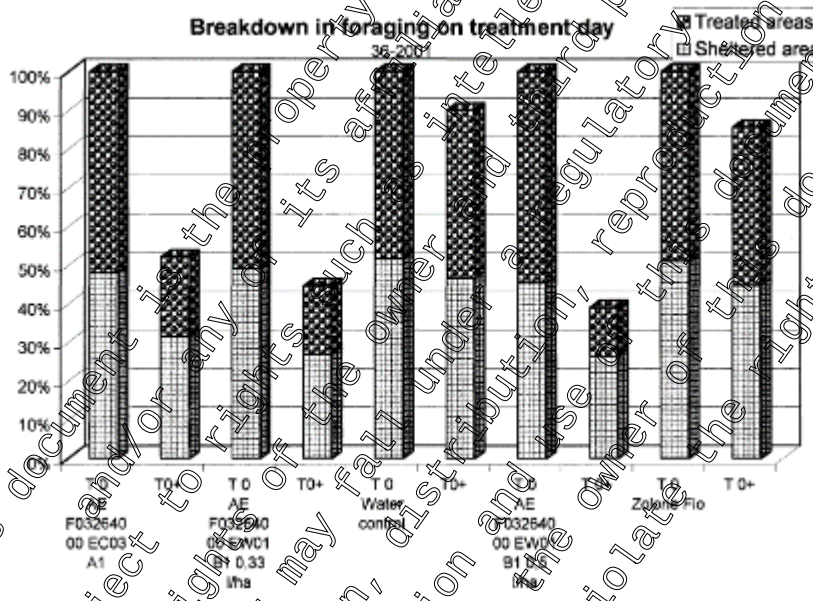


Figure 2: Breakdown of foraging on treatment day for Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 5.0 g a.s./ha, Deltamethrin EW 15 (AEF032640 00 EW01 B1 - 0.5 l/ha) at 7.5 g a.s./ha, Deltamethrin EW 15 (AEF032640 00 EW01 B106 - 0.33 l/ha) at 5.0 g a.s./ha, the reference group (Zolone Flo) and for the water control group

T0: before product application

T0+: after product application

Unfortunately unreliable data were collected on the day after treatment (1DAT) because of cold and cloudy weather all day long so that bees activity was insufficient. At 2DAT foraging increased in the three tunnels that received the study items. Then at 3DAT, foraging activity increased again in the five tunnels to reach scarcely levels of the pre treatment phase. Product applications had no longer impact on this foraging parameter.

First of all, no impact was observed in the water control tunnel or in the reference tunnel. This confirmed the very low effect of the Zolone Flo formulation on bee activity. This was shown on the graph with similar repartition of foraging bees on both sheltered and treated areas. On the contrary, items formulations induced an immediate effect on this activity, as the level diminished quickly, especially on treated zones, and this can be considered as a repulsive effect.

Colony assessment

Very few differences concerning the structure of the colonies were observed between the two visits. This state of the reserves and proportions of the brood stayed mainly stable for the different colonies. Only the colony in the tunnel where Deltamethrin EW 15 was used at 0.5 L/ha presented very less brood at the end (- 5 frames), with a very small increase in food storage (+1 frame). Despite of recorded mortalities during trial, populations remained quite stable in all tunnels between the two apiarist visits. Deaths of bees were compensated by the emergence of new bees on the brood frames, which explains this stability of bee populations.

Behaviour of the Bees

Bee colonies underwent momentary stress following introduction in the tunnels. Older foragers continually hurt themselves on the net looking to get away from the hive on a stationary or circular flight at a few meters high in order to locate themselves in the space. The volume of a tunnel represents sufficient flight space but it was nevertheless confined and colonies adapt to these experimental conditions after the first recordings.

During spraying, foraging bees flew away over treated plots when the boom passed and came down again a little further away. No aggressiveness of any frenetic bumbling was noted. However, in the tunnels with the test items a few clinical signs occuured in the hour following product application. This signs were observed in the afternoon but not the next following days.

In the standard tunnel, a characteristic Zolone smell appeared after treatment and remained for several hours. A few intoxication signs appeared and were more frequent by the end of the day, either on next days (2DAT and 3DAT).

Activity at the hive entrance was normal in all tunnels. No bee clutters were observed on the nets or at the hive entrance and no fleeing events were observed in any of the tunnels.

Clinical intoxication symptoms: In Deltamethrin EC 25, Deltamethrin EW 15 and Zolone Flo tunnels, some bees were falling down on the ground after treatment and showed typical intoxication signs. In these tunnels, intoxicated bees fell on the plastic surface of the lanes, walked in a difficult way alternating periods of sluggishness and frenzy. Such a bee rolled over itself and appeared too heavy when trying to lift off. Its fore legs then its hind legs and abdomen appeared to be paralysed. The bee died in arrange from a few minutes to a few hours.

Conclusion:

Overall conditions for conducting this experimental phase of the scheme were favourable to bee activity. Climatic and crop conditions were satisfactory. The different observed parameters agree with the collected data.

During the treatment phase, Deltamethrin EC 25 and Deltamethrin EW 15 had an impact on bees deaths compared to the water control and Zolone Flo.

Experimental conditions of the study were quite strict, including confinement and product application carried out during intense foraging activity, on very attractive plots.

The impact of Deltamethrin EW 15 whatever the dose used (0.33 and 0.5 L/ha) induced a small repulsive

effect and a raising mortality, yet limited to a short delay (only one day).

On the other hand the effects of the test substance Deltamethrin EC 25 in the case of this trial on phacelia crop only showed a lower and temporary increase in mortality yielding compareable total mortality rates to those recorded in the control tunnel.

*****

Report:	**KCP 10.3.1.5/08, [redacted]; 2001**
Title:	Assessment of effects on honeybees of AE F032640 00 EC03 A1 and AE F032640 00 EC11 A313 - Trial under insectproof tunnels on *Phacelia* crop
Document No:	M-205046-01-1 (Rep. No.: 35-2001)
Guidelines:	CEB 129
GLP:	yes

~~Material and Methods:~~

~~Honey bee colonies (ca 17,000 bees per hive, colonies of comparable development, brood and food store status, queens of homogeneous maternal origin) were confined in tunnels on *Phacelia* fields with additional pollen sources provided. Five days after introduction of the bees into the tunnels, application was performed. The test substance was applied at rates of 0.050 L/ha, and 0.075 L/ha, the toxic standard was Zolone Flo (500 g/L phosalone). Furthermore, a water treated control was set up. Treatment was carried out during flight activity of the bees. Endpoints observed were foraging activity, behavior, mortality, and colony development.~~

~~Findings:~~

~~Behavior of the bees was only slightly affected by the test item. Foraging activity was not influenced by the test substance. Mortality was not increased significantly by the test item; there was a slight and short-term increase of mortality after application, but overall mortality was comparable between treatment and control. The toxic standard, however, led to a longer lasting increase of mortality. Colony development was not affected by the treatment.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EC 25 (AE F032640 00 EC03 A1) content of a.s.: deltamethrin (analysed): 24.9 g a.s./L (25 g a.s./L nominal) Deltamethrin EC 100 (AE F032640 00 EC11 A313) content of a.s.: deltamethrin (analysed): 10.6 % w/w (100 g a.s./L nominal)
Batch number:	Deltamethrin EC 25: OP200859 Deltamethrin EC 100: 066/00PM
Reference item:	Zolone Flo (500 g a.s./L nominal, analysed content: 499 g a.s/L)
Test organism:	Honey bees (*Apis mellifera*) The used hives were of the DADANT 10 frames model, with one queen and approximately 16000 to 18000 bees per hive at test start. The colonies were as homogeneous as possible. The corresponding queens (Italian breed) were one year old and

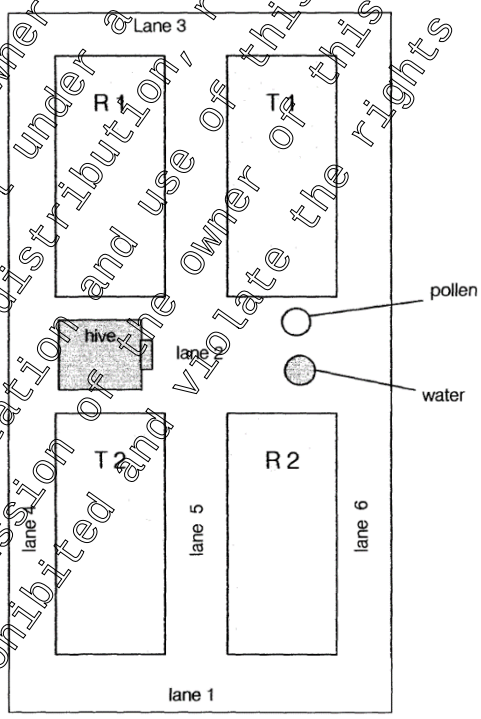
originated from one breeding line in order to guarantee uniform bee material in all treatment groups. Honeybees spread over 7 to 8 frames (of which approximately 2 to 4 frames of brood).

Source: Bee colonies came from the same apiary containing 1,200 hives allowing easy selection of swarms.

Crop: *Phacelia tanacetifolia* of the Balo variety (bee attractive crop) at flowering stage

Test location: [redacted] on a field from [redacted] France.

Test unit: Each tunnel covered an area of 140 m² (7 m x 20 m) with a roof height of approximately 3 metres. The tunnel tent frames were covered by a polyethylene mesh net (1.2 mm x 1.2 mm).
Inside the tunnels, the *Phacelia* crop was split into four plots. Each had a surface of 16 m² (2 m x 8 m). Two plots were considered as sheltered areas (R1 and R2; not treated with test item), the other two (T1 and T2) as treated areas. A beehive, a watering place and feeders with pollen were placed in each of the tunnels and supplied daily.
The interior design of a tunnel is presented below.



R1/ R2: sheltered area
T1/ T2: treated area

Application rates: Control: water
Deltamethrin EC 25: 0.2 L/ha (5 g a.s./ha) during foraging activity
Deltamethrin EC 100: 0.05 L/ha (5 g a.s./ha) during foraging activity

	Deltamethrin EC 100: 0.075 L/ha (7.5 g a.s./ha) during foraging activity Reference rate (Zolone Flo): 1.20 L/ha (0.5 kg a.s./ha) Every treatment comprised of one replicate (i.e. 1 tunnel per treatment). The spray volume was 300 to 315 L/ha in all treatment groups.
Data sampling:	The data of mortality, foraging, condition of the colonies and bee behaviour of the test item groups and the reference item group were assessed.
Data analysis:	The data of mortality, foraging activity, breakdown of foraging on treatment day and colony behaviour in the post-application period of the test item group and the reference item group were compared to the control. In each tunnel foraging bees in treated and sheltered areas are counted separately. Afterwards a mean value is derived. All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.
Deviations from the study plan:	Crop plots were damaged by a storm before treatment. The trial was stopped and carried out again on new plots. Previous data still available were not taken into account in study results. No other impact on the study.

Climatic conditions during the experiment:

The environmental conditions are shown in the following table.

Table 1: Environmental conditions during the experimental period

Prameter	Experimental period/ Day																
	30/7	31/7	1/8	2/8	3/8	4/8	5/8	6/8	7/8	8/8	9/8	10/8	11/8	12/8	13/8	14/8	15/8	16/8
Rainfall [mm]	0	0	0	6	0	1	0	3	2	33	0	3	0	0	0	0	0	0
Min T [°C]	12	15	15	18	9	10	10	15	15	11	11	4	5	7	9	11	11	5
Max T [°C]	31	31	29	26	22	20	21	19	18	20	20	20	23	27	30	33	27	24

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 2: *Phacelia* crop data

Date	Operation	Characteristics
April	Soil preparation	Herbicide application and harrowing, seedbed preparation and weed destruction
15/06/01	Plot sowing + rolling	*Phacelia tanacetifolia* at 5 kg/ha (Batch D/BN 228-0-9217)
August	Destruction	Crushing the crop on experimental plots

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EC 25 and Deltamethrin EC 100 on the honeybee, *Apis mellifera* under forced exposure conditions.

This study included five exposure groups with one replicate (tunnel) each: one tap-water treated control group, three test-item groups (1 × Deltamethrin EC 25 and 2 × Deltamethrin EC 100) and one reference item group. In all exposure groups, the crop was sprayed 5 days after set-up of the hives in the tunnels at full-flowering, during honeybees were actively foraging on the crop under confined conditions. The honeybees remained 11 days in the tunnels.

Mortality in each tunnel is recorded on a daily basis for all areas covered with plastic film, from days 4DBT to 6DAT. Moreover, the day on which product applications are carried out (0DBT) additional counts are done at the end of the day (0DAT) in order to establish possible brutal intoxication of foraging bees. The total mortality rate recorded in a tunnel for a given day results from adding up mortality rates observed in each of the six plastic rows in the tunnel.

Foraging was observed daily from 2DBT to 2DAT in all the sheltered (R1 and R2) and treated areas (T1 and T2). It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts could be shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall...etc.) This parameter was also taken into account for an additional count on D 0, during the hour following product application.

Two colony assessments were performed in the beginning and at the end of the experimentation (7DBT and 7DAT), allowing to evaluate colony development taking into account parameters like the adult bee population, the quantity and quality of the brood (different stages observed), amount of reserves and potential construction of new frames on offered wax sheets. These visits are carried out in the tunnels at dates which are as close as possible to the first and last day of confinement. However, for practical or climatic conditions, they necessarily take place within 48 hours before or after introduction of the hives in the tunnels on the one hand, and when the hives are taken out on the other hand.

Assessments of bee behaviour were carried out when products were applied and during 30 minutes following test item application in each of the tunnels. In general, this observation phase continued all over the day, between counts. Bees were especially observed for reactions and behaviour like intense flying, bee clusters, aggressiveness, beginning of intoxication etc. in each of the tunnels.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees before as well as after the applications in the control, the two test item groups and the reference item group, respectively
- Number of foraging bees/m^2 per day on all the areas (T1, T2 and R1, R2) before as well as after the applications in the control, the two test item groups and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the two test item groups and the reference item group respectively
- Colony Assessment in the beginning and at the end of experimentation

Dates of Work: 30th July to 16th August 2001

Findings:

Honey bee mortality

A summary of the daily mortality results is shown in the following table.

Table 3: Daily mortality data

Treatment	4DBT – 6th August						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Zolone Flo	6	1	0	0	0	0	21
Deltamethrin EC 25 (5.0 g a.s./ ha)	6	4	4	0	1	2	17
Deltamethrin EC 100 (5.0 g a.s./ ha)	6	5	14	2	1	21	49
Deltamethrin EC 100 (7.5 g a.s./ ha)	12	1	4	0	1	3	21
Water control	13	9	3	0	1	13	39
	3DBT - 7th August						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Zolone Flo	162	84	26	7	11	88	378
Deltamethrin EC 25 (5.0 g a.s./ ha)	63	17	7	5	1	13	106
Deltamethrin EC 100 (5.0 g a.s./ ha)	73	9	12	1	6	25	126
Deltamethrin EC 100 (7.5 g a.s./ ha)	47	8	9	4	7	25	100
Water control	72	24	7	3	3	58	167

	2DBT - 8th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	88	49	21	4	10	41	213
Deltamethrin EC 25 (5.0 g a.s./ ha)	40	26	13	1	1	29	110
Deltamethrin EC 100 (5.0 g a.s./ ha)	41	7	12	1	8	14	83
Deltamethrin EC 100 (7.5 g a.s./ ha)	52	8	13	0	0	18	91
Water control	58	15	14	3	5	38	133
	1DBT - 9th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	495	59	40	17	9	185	805
Deltamethrin EC 25 (5.0 g a.s./ ha)	137	13	7	7	6	32	202
Deltamethrin EC 100 (5.0 g a.s./ ha)	210	8	17	7	4	46	292
Deltamethrin EC 100 (7.5 g a.s./ ha)	142	7	10	4	4	25	192
Water control	280	19	15	9	10	97	430
	0DBT - 10th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	335	66	52	20	25	136	634
Deltamethrin EC 25 (5.0 g a.s./ ha)	116	15	12	19	2	28	191
Deltamethrin EC 100 (5.0 g a.s./ ha)	233	9	12	19	13	12	298
Deltamethrin EC 100 (7.5 g a.s./ ha)	111	4	9	9	6	13	152
Water control	204	2	12	13	7	62	300
	0DAT - 10th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	260	54	57	46	24	116	557
Deltamethrin EC 25 (5.0 g a.s./ ha)	86	8	16	41	3	13	167
Deltamethrin EC 100 (5.0 g a.s./ ha)	191	16	25	24	9	32	297
Deltamethrin EC 100 (7.5 g a.s./ ha)	124	3	21	12	3	19	184
Water control	109	11	7	14	7	29	177
	1DAT - 11th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	162	39	101	31	20	94	447
Deltamethrin EC 25 (5.0 g a.s./ ha)	113	13	60	81	7	20	294
Deltamethrin EC 100 (5.0 g a.s./ ha)	157	15	97	61	6	46	382
Deltamethrin EC 100 (7.5 g a.s./ ha)	132	22	95	47	2	26	324
Water control	47	10	21	12	11	19	120
	2DAT - 12th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	244	81	230	104	33	185	877
Deltamethrin EC 25 (5.0 g a.s./ ha)	61	20	66	73	7	22	249
Deltamethrin EC 100 (5.0 g a.s./ ha)	135	31	140	79	15	67	467
Deltamethrin EC 100 (7.5 g a.s./ ha)	80	22	135	47	12	33	329
Water control	75	14	118	39	8	46	300
	3DAT - 13th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	184	78	150	47	13	135	607
Deltamethrin EC 25 (5.0 g a.s./ ha)	30	7	31	27	7	12	114
Deltamethrin EC 100 (5.0 g a.s./ ha)	100	17	77	47	10	20	271
Deltamethrin EC 100 (7.5 g a.s./ ha)	61	5	49	32	5	17	169
Water control	57	8	67	25	5	41	203
	4DAT - 14th August						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Zolone Flo	lane 1	63	121	55	8	113	527
Deltamethrin EC 25 (5.0 g a.s./ ha)	167	11	17	30	2	9	102
Deltamethrin EC 100 (5.0 g a.s./ ha)	33	17	52	47	10	28	263
Deltamethrin EC 100 (7.5 g a.s./ ha)	109	5	27	27	4	11	145

Water control	71	14	61	51	7	45	264
	5DAT – 15th August						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Zolone Flo	145	76	159	61	16	89	546
Deltamethrin EC 25 (5.0 g a.s./ ha)	47	8	41	32	4	12	144
Deltamethrin EC 100 (5.0 g a.s./ ha)	125	22	71	44	7	40	309
Deltamethrin EC 100 (7.5 g a.s./ ha)	82	17	70	18	4	38	229
Water control	77	11	82	24	3	72	269
	6DAT – 16th August						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Zolone Flo	215	67	30	40	18	96	461
Deltamethrin EC 25 (5.0 g a.s./ ha)	77	10	10	22	17	20	156
Deltamethrin EC 100 (5.0 g a.s./ ha)	155	18	22	21	13	51	280
Deltamethrin EC 100 (7.5 g a.s./ ha)	122	9	24	31	11	56	243
Water control	165	9	23	15	5	98	315

DBT: days before treatment
DAT: days after treatment

A figure of the total mortality is displayed in the figure below.

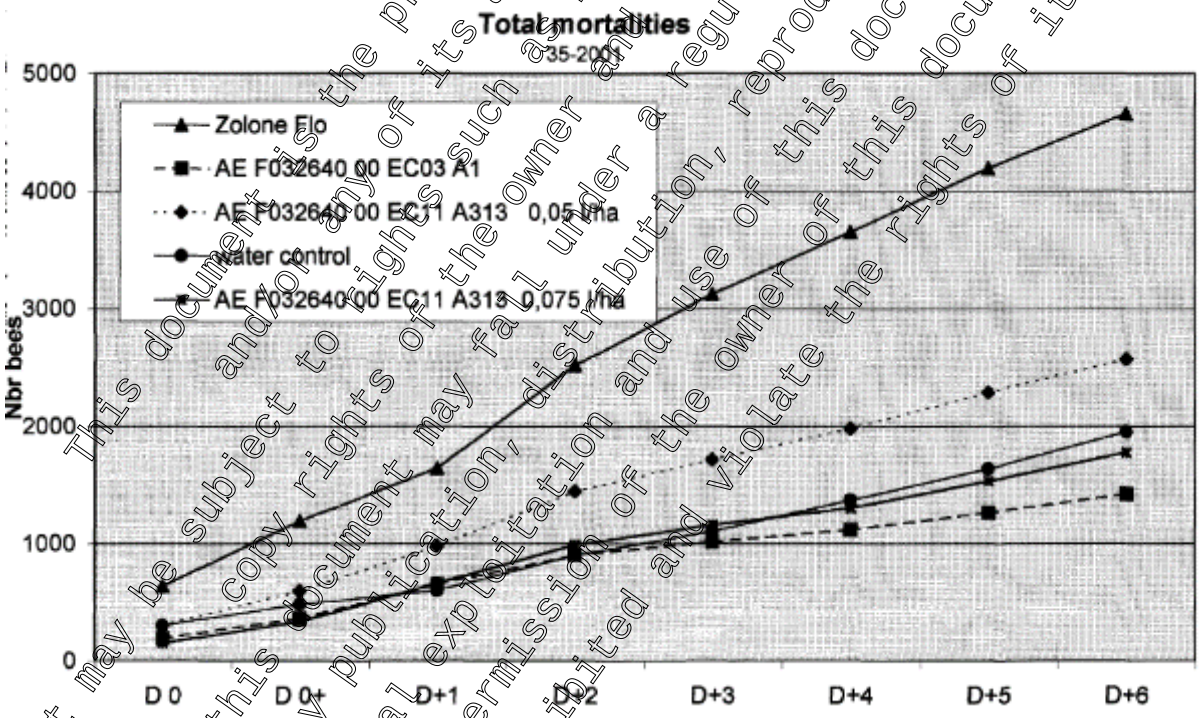


Figure 1: Total mortalities during study period for the reference group, Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 0.2 L/ha (5 g a.s./ha), Deltamethrin EC 100 (AE F032640 00 EC11 A313) at 0.05 L/ha (5 g a.s./ha) and at 0.075 L/ha (7.5 g a.s./ha) as well as for water control group

D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+6: 1 to 6 days after treatment

The day after the treatment, the test items Deltamethrin EC 25 and Deltamethrin EC 100 at two different rates and the reference item (Zolone Flo) showed an increasing mortality whereas the water control tunnel showed a regular evolution. In the control tunnel (treated with water) the colony was not disturbed by the treatment. Mortality rates recorded vary very few along the week.

At 1DAT the three test items and the reference item (Zolone Flo) induced the same peak of mortality. The peak in Zolone Flo seemed to be higher but the mortality rate in this tunnel was even higher before treatment. Deltamethrin EC 25 and Deltamethrin EC 100 formulations showed a peak at 1DAT but this phenomenon was very brief. From D+2 the mortality rates recorded in these tunnels literally drops. At 3DAT the level was comparable with the one from the pre-treatment phase and remained very low until the end of the test.

The total mortalities trends in the tunnel treated with Deltamethrin EC 25 and with Deltamethrin EC 100 at 0.075 L/ha were comparable to that of the water control tunnel. The mortality rate in the tunnel treated with Deltamethrin EC 100 at 0.05 L/ha was higher than the three precedent tunnels but much lower than the mortality rate in the Zolone Flo tunnel.

Honey bee flight intensity

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following tables.

Table 4: Foraging data: Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 0.2 L/ha (5 g a.s./ha)

AE F032640 00 EC03 A1		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 8th 01	14H30	69	45	25	36	42	37	38	57	88	87	5,5	5,4
D-2	16H00	102	68	31	51	48	57	67	74	126	123	7,9	7,7
											105		6,6
August 9th 01	11H00	55	73	48	19	47	43	62	53	98	103	6,1	6,4
D-1	12H00	97	76	48	54	46	71	76	78	138	136	8,6	8,5
	14H30	117	102	65	85	94	84	79	130	185	194	11,5	12,1
											144		9,0
August 10th 01	11H30	80	56	40	42	78	55	62	62	109	129	6,8	8,0
	13H30	70	71	39	40	64	53	47	52	110	108	6,9	6,8
D 0											118		7,4
	15H00	119	107	69	72	88	63	47	67	184	133	11,5	8,3
	16H00	145	138	89	89	169	98	111	143	231	261	14,4	16,3
	17H00	148	141	99	108	182	124	136	150	248	296	15,5	18,5
											230		14,4
August 11th 01	10H30	98	112	89	70	91	127	124	76	185	209	11,5	13,1
D+1	11H30	99	109	103	79	95	116	137	100	195	224	12,2	14,0
	13H00	129	125	108	84	114	150	158	111	228	267	14,3	16,7
											233		14,6
August 12th 01	10H30	138	128	76	93	126	116	120	99	218	231	13,6	14,4
D+2	11H30	155	106	103	125	128	117	133	148	245	263	15,3	16,4
	12H30	164	157	113	129	172	118	140	151	282	291	17,6	18,2
											261		16,3

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator.
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 et T2.
Nb bees/m^2 : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m^2.
D-2/ D-1: 2/ 1 days before treatment
D0: day of treatment
D+1/ D+2: 1/ 2 days after treatment

Table 5: Foraging data: Deltamethrin EC 100 (AE F032640 00 EC11 A313) at 0.05 L/ha (5 g a.s./ha)

AE F032640 00 EC11 A313 0,05 l/ha		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 8th 01	14H30	81	57	41	23	65	42	72	51	101	115	6,3	7,2
D-2	16H00	87	78	58	40	96	56	91	79	132	161	8,2	10,1
											138		8,6
August 9th 01	11H00	52	60	43	62	65	83	88	85	109	161	6,8	10,0
D-1	12H00	70	72	55	60	78	90	105	81	128	177	8,0	11,1
	14H30	123	92	76	64	121	105	124	125	178	238	11,1	14,8
											192		12,0
August 10th 01	11H30	71	56	43	39	81	48	82	46	105	129	6,5	8,0
	13H30	58	61	52	22	79	70	61	55	97	133	6,0	8,3
D 0											131		8,2
	15H00	96	106	68	39	122	80	87	90	155	190	9,7	11,8
	16H00	114	109	85	62	146	100	124	148	185	260	11,6	16,2
	17H00	141	127	121	64	164	120	176	173	227	317	14,2	19,8
											255		15,9
August 11th 01	10H30	73	107	80	105	97	130	158	133	183	259	11,4	16,2
D+1	11H30	96	105	71	78	89	118	114	106	175	214	10,9	13,3
	13H00	100	109	86	116	104	147	141	151	206	272	12,8	17,0
											248		15,5
August 12th 01	10H30	127	81	120	78	127	85	147	142	203	251	12,7	15,7
D+2	11H30	123	108	135	89	141	98	167	151	228	279	14,2	17,4
	12H30	138	97	149	96	150	127	173	180	240	315	15,0	19,7
											281		17,6

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 et T2.
Nb bees/m²: number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-2/ D-1: 2/ 1 days before treatment.
D0: day of treatment.
D+1/ D+2: 1/ 2 days after treatment.

Table 6: Foraging data: Deltamethrin EC 100 (AE F032640 00 EC11 A313) at 0.075 L/ha (7.5 g a.s./ha)

AE F032640 00 EC11 A313 0,075 l/ha		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 8th 01	14H30	70	50	91	63	51	60	100	76	137	144	8,6	9,0
D-2	16H00	70	47	100	82	65	60	113	88	150	163	9,3	10,2
											153		9,6
August 9th 01	11H00	56	74	83	93	67	45	86	97	153	148	9,6	9,2
D-1	12H00	87	76	78	108	66	92	131	122	175	206	10,9	12,8
	14H30	121	123	114	106	100	120	154	158	232	266	14,5	16,6
											206		12,9
August 10th 01	11H30	76	65	39	37	49	49	66	59	109	112	6,8	7,0
	13H30	77	76	125	71	91	73	87	108	175	180	10,9	11,2
D 0											146		9,1
	15H00	106	133	148	124	107	110	130	135	256	241	16,0	15,1
	16H00	121	124	160	133	135	115	149	174	269	287	16,8	17,9
	17H00	103	126	114	91	89	91	170	152	217	251	13,6	15,7
											260		16,2
August 11th 01	10H30	92	94	131	142	120	110	155	178	230	278	14,3	17,3
D+1	11H30	116	111	133	141	135	112	189	158	251	297	15,7	18,6
	13H00	167	110	151	188	148	152	154	178	308	316	19,3	19,8
											297		18,6
August 12th 01	10H30	148	133	184	157	145	160	220	186	311	356	19,4	22,2
D+2	11H30	131	112	174	177	134	147	193	162	297	318	18,6	19,9
	12H30	134	155	186	159	178	163	213	194	317	374	19,8	23,4
											349		21,8

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 et T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t)/ 2] /16 Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-2/ D-1: -2/ 1 days before treatment
D0: day of treatment
D+1/ D+2: 1/ 2 days after treatment

Table 7: Foraging data: Reference item (Zolone Flo)

Zolone Flo		raw data / nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
August 8th 01	14H30	46	25	25	42	36	34	38	32	69	70	4,3	4,4
D-2	16H00	54	43	26	50	54	35	57	30	87	88	5,4	5,5
											79		4,9
August 9th 01	11H00	33	26	38	17	44	41	27	30	57	71	3,6	4,4
D-1	12H00	46	29	38	52	48	40	54	46	83	94	5,2	5,9
	14H30	73	66	50	84	81	65	69	76	137	146	8,5	9,1
											104		6,5
August 10th 01	11H30	31	31	31	37	46	26	39	40	65	76	4,1	4,7
	13H30	44	45	39	51	49	38	38	43	90	84	5,6	5,3
D 0											80		5,0
	15H00	56	65	48	84	75	58	55	60	127	124	7,9	7,8
	16H00	71	79	83	113	104	78	77	91	173	175	10,8	10,9
	17H00	59	41	64	44	76	45	75	53	104	125	6,5	7,8
											141		8,8
August 11th 01	10H30	56	66	63	41	51	56	72	54	113	122	7,1	7,6
D+1	11H30	71	60	94	63	83	78	75	76	144	156	9,0	9,8
	13H00	69	78	90	67	88	100	77	101	152	183	9,5	11,4
											154		9,6
August 12th 01	10H30	84	69	73	83	81	67	84	79	155	156	9,7	9,7
D+2	11H30	92	80	87	94	108	90	114	113	177	213	11,0	13,3
	12H30	80	77	96	83	129	82	127	101	168	220	10,5	13,7
											196		12,2

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 et T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] / 16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-2/ D-1/ D-2/ 1 days before treatment
D0: day of treatment
D+1/ D+2: 1/ 2 days after treatment

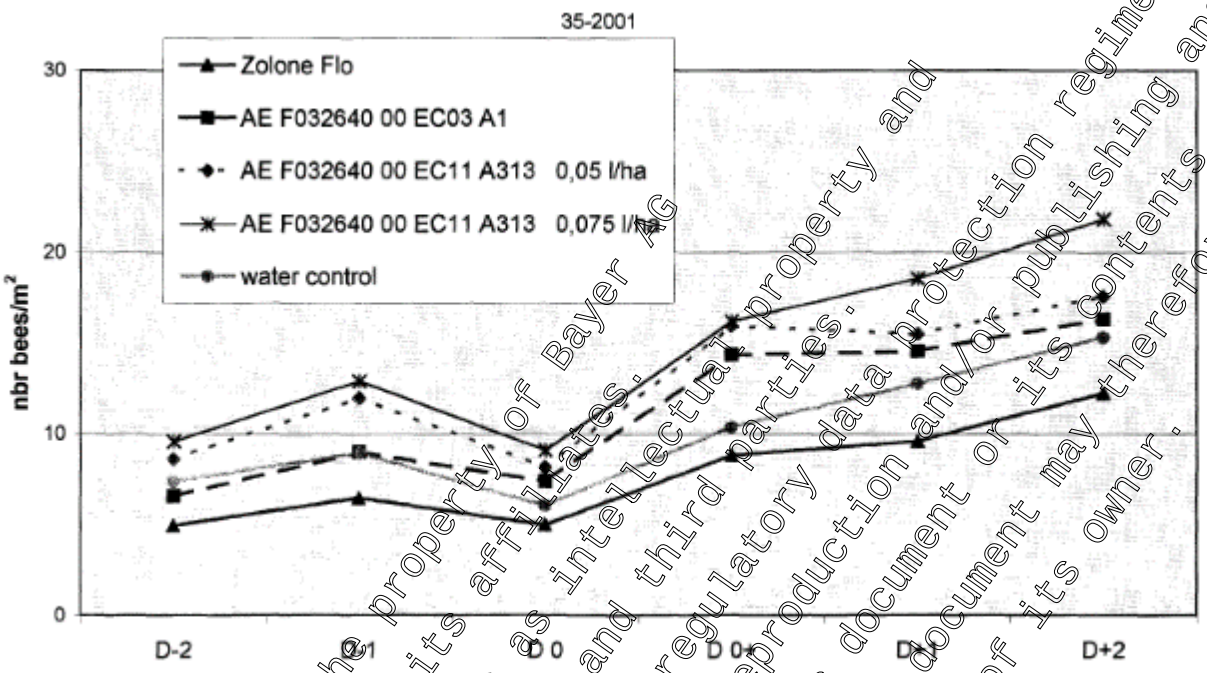


Figure 2: Mean daily foraging activity on treated areas for the reference group (Zolone Flo), Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 0.2 L/ha (5 g a.s./ha), Deltamethrin EC 100 (AE F032640 00 EC11 A313) at 0.05 L/ha (5 g a.s./ha) and at 0.075 L/ha (7.5 g a.s./ha) as well as for water control group

D0: 0 days before treatment
D0+: 0 days after treatment
D+1 / D+2: 1 / 2 days after treatment
D-2 / D-1: 2/ 1 days before treatment

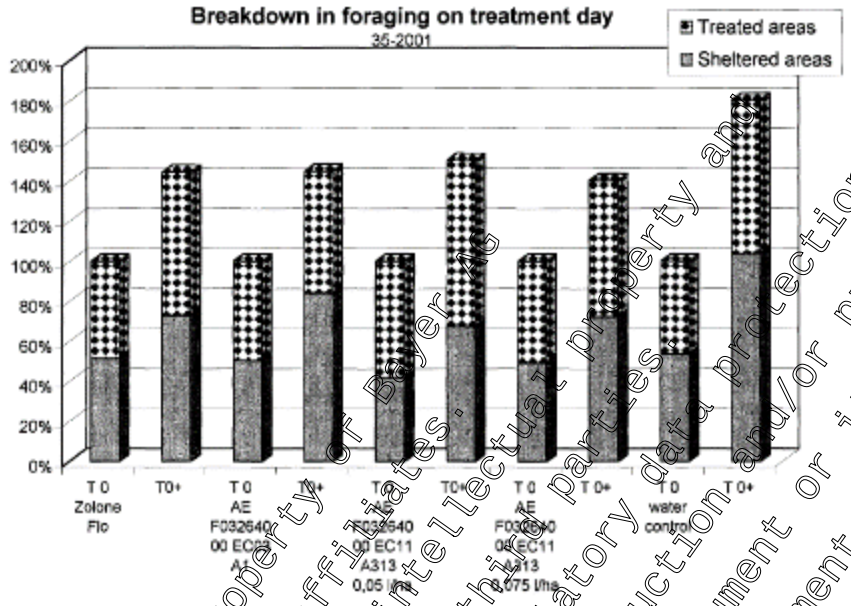


Figure 3: Breakdown in foraging on treatment day for the reference group, Deltamethrin EC 25 (AE F032640 00 EC03 A1) at 0.2 L/ha (5 g as./ha), Deltamethrin EC 100 (AE F032640 00 EC11 A313) at 0.05 L/ha (5 g a.s./ha) and at 0.075 L/ha (7.5 g a.s./ha) as well as for water control group in treated and untreated areas
T0: before product application
T0+: after product application

Foraging activity increased strongly after treatment for all tunnels. This evolution is similar for the 5 tunnels despite of the treatment phase.

All the tunnels showed an increasing foraging activity on sheltered and treated areas, this explained levels over 100%. Foraging activity increased as climatic conditions were better in the afternoon. So there was not any repulsive effect for any test item. There was no significant difference between the five tunnels.

Colony assessment

There were some differences concerning the structure of the colonies between the two visits. This state of the reserves and proportions of the brood remained stable for colonies in the test item Deltamethrin EC 25 tunnel and water control tunnel. On the contrary, colonies from both test item Deltamethrin EC 100 tunnels as well as standard tunnel presented less brood and more storage at the end of the experimental phase. Despite of recorded mortalities during trial, populations remained quite stable in all tunnels between the two apiarist visits. Deaths of bees were compensated by the emergence of new bees on the brood frames, which explained the stability of bee populations.

Behaviour of the bees

Bee colonies had momentary stress following introduction in the tunnels. Older foragers continually hurt themselves on the net looking to get away from the hive on a stationary or circular flight at a few meters high in order to locate themselves in the space. The volume of a tunnel represented sufficient flight space but it was nevertheless confined and colonies adapted to these experimental conditions after the first recordings.

During spraying, foraging bees flew away over treated plots when the boom passed and came down again a little further away. No aggressiveness or any frenetic bumbling was noted. However, in the three tunnels with the test items a few clinical signs occurred in the hour following product application. This signs were observed in the afternoon but not the next following days.

In the standard tunnel, a characteristic Zolone smell appeared after treatment and remained for several hours. A few intoxication signs appeared and were more frequent by the end of the day, either on next days (1DAT to 3DAT).

Activity at the hive entrance was normal in all tunnels. No bee clutters were observed on the nets or at the hive entrance and no fleeing events were observed in any of the tunnels.

Clinical intoxication symptoms: In Deltamethrin EC 25, Deltamethrin EC 100 and Zolone Flo tunnels, some bees were falling down on the ground after treatment and showed typical intoxication signs. Bees walked in a difficult way alternating periods of sluggishness and frenzy. Such a bee rolled over itself and appeared too heavy when trying to lift off. Its fore legs then its hind legs and abdomen appeared to be paralysed. The bee died in arrange from a few minutes to a few hours.

Conclusion:

Overall conditions for conducting this experimental phase of the scheme were favourable to bee activity. Climatic and crop conditions were satisfactory. The different observed parameters agree with the obtained data.

During the treatment phase, Deltamethrin EC 25, Deltamethrin EC 100 and Zolone Flo had an impact on bees deaths compared to the water control.

Experimental conditions of the study were quite strict, including confinement and product application carried out during intense foraging activity, on very attractive plots. Only the use of Zolone Flo induced a high mortality. The effect of the test items Deltamethrin EC 25 and Deltamethrin EC 100 in the case of this trial on a phacelia crop only showed a temporary increase in mortality yielding comparable total mortality rates to those recorded in the control tunnel.

*****

Report:	**KCP 10.3.1.5/09, [redacted]; 2000**
Title:	Toxicity to honeybee *Apis mellifera* L. (semi-field test) following the EPPO Guideline No. 170 (1992), Codes: AE F032640 00 EC03 B003 / AE F032640 00 EG06 A107
Document No:	M-197723-01-1 (Rep. No.: 991018103)
Guidelines:	EPPO 170
GLP:	yes

~~**Material and Methods:**~~

~~In three replicates, each time with a duration of 7 days, the toxicity of the test substances AE F032640 00 EC03 B003 (=EC 25) and AE F032640 00 EG06 A107 (=EG 06) to the honeybee *Apis mellifera* L. was examined in a semi-field test. The test substances were applied at the following doses: EC 25: 12.5 g a.s./ha = 0.5 L product/ha in 300 L/ha of water and EG 06: 12.5 g a.s./ha = 0.2 kg product/ha in 300 L/ha of water. The influence of the EC 25 and the EG 06 was evaluated by comparing the effect~~

of the two substance variants to the effect of the control variant and the toxic standard variant regarding the following observations:

- Mortality at the edge of the treated area and in the bee traps
- Foraging activity (number of forager bees/m² flowering *Phacelia* crop)
- Behaviour of the bees on the crop and around the hive
- Development of the bee brood

Findings

Effect on honey bee mortality and foraging activity:

For the control variant the average number of bees in the dead bee trap and around the flowering *Phacelia* showed no significantly increased mortality observed on day 0 (application), day 1, 2 and 7 compared to day -1 (before application). For the EC 25 treated variant, statistically significant more dead bees were found on day 0 (after application) when compared to day -1 (before application). The mortality on the assessment days 1, 2 and 7 compared to day 0 was increased but not statistically significant. The EG 06 treated variant showed statistically significant more dead bees found on day 0 (after application) when compared to day -1 (before application). The mortality on the assessment days 1, 2 and 7 compared to day 0 showed an increase, which was not statistically significant. No significant differences concerning flight activity were observed for all variants 1 hour before application (average number of bee/m² between 15.3 and 18.6). For the control variant no significant decrease in flight activity was observed for all assessment days after application. The flight activity of the treated variants was compared directly to control because the flight activity in all treated variants was higher than 100 % when compared to control before application (on day 0). For the EC 25 treated variant the flight activity was significant decreased on assessment day 0 immediately after application. The flight activity was decreased to a 35.7 % level compared to control bee flight activity. Starting with assessment day 1 the flight activity increased again slowly from a 45.6 % up to a 93.9 % flight activity level compared to control during the days 3-7. Bees with acute toxic reactions were found on the crop and on the entrance of the bee hive shortly after application. The most affected bees were found on the ground showing apathy, with discoordinated movements lying on their back. On assessment days 1-7 no behavioural anomalies of the bees were observed compared to control. The EG 06 treated variant showed statistically significant decreased flight activity of a 37.8 % level compared to control on assessment day 0 immediately after application. From assessment day 1 the flight activity increased again slowly from a 54.4 % up to a 89.3 % level compared to control during the days 3-7. On the crop and at the entrance of the hive bees with acute toxic reactions were found only shortly after application. The affected bees were found on the ground and at the hive entrance with discoordinated movements lying on their back or showing apathy. On assessment days 1-7 no behavioural anomalies of the bees were observed compared to control.

Effects on honey bee brood development:

The bee brood was assessed before application and on day 7 after application. Based on these assessments the percent area occupied by eggs, larvae, sealed cells, honey, pollen and empty cells was determined comparing day 7 after application with the pre-treatment level. The brood development of the treated variants was compared directly to control. For the control variant the following brood development compared to the pre-treatment level was assessed after test termination on day 7 after application: 82% eggs and 87% sealed cells. The number of larvae compared with the pre-treatment level was 5.6% and unexpected low. The queen bee of each replicate was available and external healthy on assessment day 7 after application. For the EC 25 treated variant the following bee brood development compared to the pre-treatment level was assessed after test termination on day 7 after

~~application: 180% eggs, 17% larvae and 123% sealed cells. Based on these assessments no significant inhibition of brood development was observed. The EG 06 treated variant showed the following brood development compared to the pre-treatment level after test termination on day 7 after application: 53% eggs, 14% larvae and 150% sealed cells. The occupied egg area was with 53% compared to the pre-treatment level significant lower as observed in the control. Compared to control, no influence on the bee brood development including larvae and sealed cells was observed.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EC 25 G (AE F032640 00 EC03 B003), content of a.s.: 2.76 % w/w (25 g a.s./L nominal; density: 0.893 g/cm^3) Deltamethrin EG 6.25 (AE F032640 00 EG06 A107), content of a.s.: 6.14 % w/w (6.25 % nominal)
Batch number:	Deltamethrin EC 25: 7CD11324 Deltamethrin EG 6.25: 8FES0248
Reference item:	Dimethoate EC 400 (400 g a.s./L nominal; analysed content: 395.7 g a.s./L)
Test organism:	*Apis mellifera carnica* L. Per test unit a bee hive of a Nucleus colony with 3 combs, approximately 5000 bees, the queen and all stages of brood.
Source:	[redacted]
Crop:	*Phacelia tanacetifolia* at flowering
Test location:	[redacted]
Test unit:	Tents with a size of 3.5 m × 4.5 m × 2 m covered with a special gauze (mesh width: 2 mm × 2 mm).
Application rates:	Control (C): deionized water Treatment rate (Deltamethrin EC 25): 12.5 g a.s./ha during foraging activity Treatment rate (Deltamethrin EG 6.25): 12.5 g a.s./ha Reference rate (Dimethoate EC 400): 1 L/ha (400 g a.s./ha) Every treatment comprised of three replicates (i.e. 3 tunnel per treatment). The spray volume was 300 L/ha in all treatment groups.
Data sampling:	Data for mortality, foraging activity (flight intensity), behaviour of the bees and bee brood (incl. conditioning of queen) were assessed.
Data analysis:	All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.
Deviations from the study plan:	No deviation mentioned.

Climatic conditions during the experiment:

The experiment was conducted under climatic conditions that favoured intensive foraging activity. The environmental conditions are shown in the following table.

Table 1: Weather conditions during the exposure period

Date	Air temperature (°C)		Rainfall (mm)		Rel. air humidity (%)	
	min	max	min	max	min	max
beehive placed in the field cage (replicates 1 + 2) 25.08. - 29.09.99	15.9	19.4	0.0	2	45	78
application/ start of the test (replicates 1 + 2) 30.08. - 6.09.99	15	19.3	0.0	1.4	49	67
beehive placed in the field cage (replicate 3) 7.09. - 9.09.99	18	20.2	0.0	5.7	60	71
application/ start of the test (replicate 3) 10.09. - 17.09.99	15.5	21.4	0.0	0.0	43	56

Pesticide history of the field site:

No pesticide history of the field was stated in the study report.

The effects of Delthamethrin EC 25 and Delthamethrin EG 6.25 were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the EPPO Guideline No. 170 (1992)

The purpose of the study was the examination of the side effects of the two substances Delthamethrin EC 25 and Delthamethrin EG 6.25 on honeybees, *Apis mellifera*, under semi-field conditions.

This study included four exposure groups with three replicates (tunnels) each: one deionized water treated control group, two test-item groups (Delthamethrin EC 25 and Delthamethrin EG 6.25) and one reference item group (dimethoate). The crop was sprayed five days (replicate 1 + 2) and 3 days (replicate 3), respectively, after bee hives were transferred to test units for acclimatisation.

In all exposure groups, the crop was flowering. The honeybees remained 7 days in the tents after application. Five days (replicate 1 and 2) or 3 days (replicate 3), respectively, before application the bee boxes were filled with a 3-comb-beehive. One test beehive was placed in each tent near the entrance.

Assessment of mortality (dead bees in the bee trap and around the Phacelia) and foraging activity (flight intensity) was recorded daily during 5 or 3 days before and 7 days after test item application. Bee brood (evaluation of the current brood status of each comb, number of eggs, larvae and pupae in %) was assessed one day before (1DBT) and seven days after application (7DAT). Additionally, condition of the queen was assessed. Furthermore, behaviour of bees in hive and foraging bees was evaluated for poisoning symptoms or any anomalous behaviour in comparison with untreated bees.

The influence of the test items were evaluated by comparing the results obtained in the test item treatment groups to those of the control and the reference item group.

The following endpoints were assessed:

- Total and mean number of dead bees before as well as after the application in the control, in the treatment and reference item groups, respectively.
- Flight intensity (mean number of forager bees/m^2) before as well as after the applications in the control, in the treatment and reference item groups, respectively.
- Behaviour of the bees in the hive and of the foraging bees before as well as after the application in the control, in the treatment and reference item groups, respectively.
- Condition and development of the bee brood before as well as after the application in the control, in the treatment and reference item groups, respectively

Dates of Work: 30th August to 17th September 1999

Findings:

Honey Bee Mortality:

A summary of the average daily mortality and % mortality compared to day before application are shown in table 2. Results of three replicates of each treatment group were summed up.

Table 2: Average mortality and % mortality compared to day before application

Average number of dead bees (dead bee trap + bees collected around Phacelia)				
	Control	**Reference substance**	**Delthamethrin EC 25 @12.5 g a.s./ha**	**Delthamethrin EG 6.25 @12.5 g a.s./ha**
1DBT	22.0	23.3	21.6	20.7
0DAT	14.7	410.3*	143.3*	103.0*
1DAT	17.6	186.0	45.6	41.3
2DAT	18.3	152.5	37.6	34.0
3DAT to 7DAT	25.4	74.6	28.1	30.1
% pre-treatment level (compared to day before application)				
0DAT	66.8	1760.9	663.4	497.6
1DAT	80.0	798.3	211.1	199.5
2DAT	83.2	654.5	174.1	164.3
3DAT to 7DAT	115.5	320.2	130.1	145.4

*statistically significant ($\alpha = 0.05$) compared to the pre-treatment level/replicate
DBT: days before treatment
DAT: days after treatment

Table 3: Daily assessment of mortality (number of dead bees in the bee trap and around the Phacelia)

		5DBT	4DBT	3DBT	2DBT	1DBT	0DBT	0DBT -1h	0DAT	1DAT	2DAT	3DAT	4DAT	5DAT	6DAT	7DAT	3-7DAT
		Control															
R1	T	34	62	11	0	0	0	1	1	0	0	0	1	2	4	4	11
	E	10	7	2	1	7		5	2	3	6	6	5	21	17	13	62
R2	T	59	113	56	0	3	0	0	1	0	1	0	0	2	2	1	5
	E	19	26	8	9	20		6	7	9	7	9	15	31	35	25	115
R3	T			18	3	3	3	0	1	0	0	3	0	0	5	1	9
	E			46	52	33		14	32	41	41	40	41	39	36	23	179
		Reference															
R1	T	19	25	0	12	0	1	0	109	100	68	37	22	8	4	4	75
	E	32	14	3	13	11		4	142	43	73	39	48	59	46	59	251
R2	T	33	91	25	14	4	2	0	112	82	31	17	27	7	24	20	95
	E	31	32	0	6	7		4	104	54	50	29	51	57	64	27	278
R3	T			110	25	4	3	0	100	84	47	9	6	6	4	5	30
	E			96	115	44		30	659	195	189	120	98	84	58	31	391
		Delthamethrin EC 25 @12.5 g a.s./ha															
R1	T	21	6	0	1	11	8	1	9	6	2	0	1	1	0	2	4
	E	13	2	4	8	4		4	73	15	15	7	10	19	22	18	76
R2	T	49	75	5	29	3	19	1	9	3	1	1	2	5	2	3	13
	E	52	36	8	8	7		9	98	17	11	15	23	23	37	20	118
R3	T			32	32	5	0	1	10	4	3	1	0	3	3	2	9
	E			98	79	35		25	231	92	81	35	43	66	39	19	202
		Delthamethrin EG 6.25 @12.5 g a.s./ha															
R1	T	17	55	14	11	8	1	10	1	5	4	2	4	4	5	19	
	E	42	26	4	8	4		8	44	16	16	17	13	24	25	27	106
R2	T	34	83	23	14	2	2	0	31	1	0	0	2	0	7	2	11
	E	24	28	5	14	6		9	60	17	23	12	19	35	38	35	139
R3	T			65	68	8	4	0	6	3	3	0	1	2	1	1	5
	E			58	89	31		15	198	86	55	25	38	41	46	22	172

T: Trap; E: Edges; R1-R3: Replicate; DBT: days before treatment; DAT: days after treatment

For the Delthamethrin EC 25, Delthamethrin EG 6.25 and reference item treated variant statistically significant more dead bees were found on 0DAT (after application) when compared to 1DBT (before application). Mortality on assessment days 1, 2 and 7 compared to day 0 showed an increase but not statistically significant mortality. The average number of dead bees decreased rapidly from 1DAT until 3-7DAT after application (211.1% to 130.1% for Delthamethrin EC 25, 199.5 % to 145.4% for Delthamethrin EG 6.25 and 798.3% to 320.2% for the reference item).

Honey Bee Flight Intensity:

The validity criterion was accomplished (foraging activity ≥ 10-12 bees/m^2 during application). A summary of the average number of bees on the flowers and an average flight activity in % compared to control are shown in the following table.

Table 4: Foraging activity during study period

Average number of bees on the flowers per m^2/field cage/day				
	Control	**Reference substance**	**Delthamethrin EC 25 @12.5 g a.s./ha**	**Delthamethrin EG 6.25 @12.5 g a.s./ha**
1DBT	15.3	16.3	18.9	18.6
0DAT	14.3	2.2	6.0	5.4
1DAT	12.5	0.0	5.7	6.8
2DAT	11.5	0.2	8.2	9.5
3DAT to 7DAT	13.1	3.9	12.3	11.7
Average flight activity (%) compared to control				
1DBT		106.5	117.6	121.6
0DAT		15.4	35.7	37.8
1DAT		0.0	45.6	54.4
2DAT		1.7	71.3	82.6
3DAT to 7DAT		29.8	93.9	89.3

DBT: days before treatment
DAT: days after treatment

Table 5: Daily assessment of flight activity on the Phacelia (number of bees/m²) in the control, reference item and the two treatment groups (Delthamethrin EC 25 G at 12.5 g a.s./ha, Delthamethrin EG 6.25 at 12.5 g a.s./ha)

Assessment of flight activity on the Phacelia (number of bees/m²)																										
Assessment on day	-5	-4	-3	-2	-1	0						1				2				3	4	5	6	7	Σ	mean/ day
variant/replicate						-1h	1h	2h	4h	6h	Σ	m	n	a	Σ	m	n	a	Σ	n	n	n	n	n		
control (R1)	6	2	5	2	10	11	20	18	17	0	55	0	3	8	11	0	8	4	12	6	10	14	10	11	51	10.2
control (R2)	8	5	4	3	8	14	18	12	14	1	45	0	6	12	18	0	10	7	17	6	14	11	12	14	57	11.4
control (R3)			14	12	21	21	24	26	17	5	72	0	22	24	46	0	18	22	40	20	22	24	3	20	89	17.8
reference (R1)	10	4	6	2	12	12	2	1	1	0	4	0	0	0	0	0	0	0	0	5	3		3	8	24	4.8
reference (R2)	9	5	4	3	10	12	1	2	0	0	3	0	0	0	0	0	0	0	0	5	2	6	2	7	22	4.4
reference (R3)			11	13	24	25	10	8	1	0	19	0	0	0	0	0	0	1	1	3	3	2	4	12	13	2.6
S 3 (R1)	12	3	7	3	9	15	2	10	7	0	19	0	6	8	14	0	6	6	12	7	10	13	10	12	52	10.4
S 3 (R2)	9	5	4	1	12	14	1	4	5	0	10	0	5	8	13	0	6	8	14	7	9	15	15	10	53	10.6
S 3 (R3)			12	14	26	25	20	10	2	0	32	0	3	4	7	0	8	15	23	10	24	21	5	16	79	15.8
S 4 (R1)	11	2	7	3	9	15	2	8	5	0	15	0	4	8	12	0	6	6	12	6	12	16	8	6	48	9.6
S 4 (R2)	10	5	5	5	8	14	1	6	4	0	11	0	4	9	13	0	10	7	17	5	8	14	12	10	49	9.8
S 4 (R3)			12	14	21	27	22	10	5	1	39	0	8	8	16	0	10	18	28	12	24	20	3	18	79	15.8

S3: AE F032640 00 EC03 B003 (0.5 l/ha) S4: AE F032640 00 EG06 A107 (0.2 kg/ha)
(R): replicate m: morning n: noon a: afternoon

T: Trap; E: Edges; R1-R3: Replicate
S3: Delthamethrin EC 25 G (AE F032640 00 EC03 B003) at 12.5 g a.s./ha
S4: Delthamethrin EG 6.25 (AE F032640 00 EG06 A107) at 12.5 g a.s./ha

No significant differences concerning flight activity were observed for all variants 1 hour before treatment. On all assessment days bees of the control variant showed normal flight and foraging activities on the crop and around the hive.

For Delthamethrin EC 25 and Delthamethrin EG 6.25 flight activity was significant decreased on assessment day 0 immediately after application. Starting with assessment day 1 the flight activity increased slowly compared to the control during 3-7DAT. For both Delthamethrin EC 25 and Delthamethrin EG 6.25 bees with acute toxic reactions were found on the crop and on the entrance of the bee hive shortly after application. The most affected bees were found on the ground and at the hive entrance showing apathy, with discoordinated movements lying on their back. On assessment days 1DAT to 7DAT no behavioural anomalies of the bees were observed compared to control.

For the reference item acute toxic reactions were found on the crop and at the entrance of the hive. The following reactions were observed: irritations, discoordinated movements and restlessness.

Development of the bee brood

A summary of the development of the bee brood is presented in the table below.

Table 6: Development of the bee brood before (b) and seven days after application for the control, reference substance, Delthamethrin EC 25 (AE F032640 00 EC03 B003) and Delthamethrin EG 6.25 (AE F032640 00 EG06 A107)

% eggs/larvae/sealed cells/honey/pollen/empty cells (average of replicate 1+2+3)																							
	Control						reference substance						AE F032640 00 EC03 B003 (0.5 l/ha)						AE F032640 00 EG06 A107 (0.2 kg/ha)					
	E	L	S	H	P	Em	E	L	S	H	P	Em	E	L	S	H	P	Em	E	L	S	H	P	Em
b	11	18	15	23	1	33	34	13	12	8	4	29	10	24	13	23	1	31	17	14	14	10	6	39
a	9	1	13	17	1	60	9	4	17	18	0	53	18	4	16	19	1	41	9	2	21	28	1	40
ptl	82	5.6	87	74	100	182	26	31	142	225	0	183	180	17	123	83	100	132	53	14	150	156	0	103

b: before application a: day 7 after application ptl: % pre-treatment level (compared to brood assessment before application)

E: eggs L: larvae S: sealed cells H: honey P: pollen Em: empty

For the Delthamethrin EC 25 treated variant no significant inhibition of brood development was observed. The queen bee of each replicate was available and healthy on assessment day 7 after application (7DAT).

For the Delthamethrin EG 6.25 treated variant, area occupied by eggs was compared to the pre-treatment level significant lower as observed in the control. Compared to control no influence on the bee brood development including larvae and sealed cells was observed. The queen be of replicate 2 + 3 was available and healthy on assessment day 7 after application. The queen bee of replicate 1 did not exist and could not be found on any comb inside the hive on day 7 after application (7DAT).

The toxic reference treatment resulted in a decreased number of eggs of 26 % compared to the pre-treatment level. The queen bee of replicate 3 did not exist and could not be found on any of the combs inside the hive on day 7 after application. All other brood assessments resulted in no significant inhibition in the percent larvae (31 %) and sealed cells (142 %) compared to the control on day 7 after application.

Conclusion:

Delthamethrin EC 25 (0.5 L product/ha) and Delthamethrin EG 6.25 (0.2 kg/ha) caused significant effects on the bee mortality in dependence on the time of assessment. The highest and statistically significant mortality in comparison to the pre-treatment mortality was observed immediately after application on day 0. Starting with day 1 after application the bee mortality decreased noticeable up to the control level observed on day 7 after application.

The foraging activity decreased significantly immediately after application for the Delthamethrin EC 25 (0.5 L product/ha) and Delthamethrin EG 6.25 (0.2 kg/ha) treatment. The foraging activity was not completely decreased up to a 0 level because during all assessments after application foraging bees were observed on the treated plants. Starting with day 1 after application the foraging activity for the Delthamethrin EC 25 (0.5 L product/ha) and Delthamethrin EG 6.25 (0.2 kg/ha) treatment reached a non-significant level compared to the control foraging activity.

For the Delthamethrin EC 25 treated variant the brood development compared to the pre-treatment level was assessed after test termination on day 7 (7DAT). There was no significant influence on brood development or queen bee behavior compared to the pre-treatment level. Compared to the control no significant influence on brood development was observed, too.

The Delthamethrin EG 6.25 treated variant showed 53 % eggs, 14 % larvae and 150 % sealed cells compred to the pre-treatment level observed on day 7 after application. The comparison of the area occupied by eggs was with 53 % of the pretreatment level lower as observed in the control.

The queen bee of replicate 1 could not be found on any comb inside the hive on day 7 after application. On comb number two of replicate 1queen cells were observed in order to produce another queen bee. However, based on this observation the queen bee in replicate 1 was not available on day 7 after application. Compared to the control treatment no negative influence on the brood development including the larvae and sealed cells was observed.

The brood development assessed before and 7 days after application resulted in no definite statement regarding the influence of Delthamethrin EC 25 (0.5 L product/ha) and the Delthamethrin EG 6.25 (0.2 kg/ha) on the brood development.

However, in consideration of the low number of eggs and developing larvae from eggs for all treatments observed on day 7 after application no significant negative influence of the Delthamethrin EC 25 and the Delthamethrin EG 6.25 treatment on the brood development could be determined when compared to control.

*****

Report:	**KCP 10.3.1.5/10, [redacted]; 2000**
Title:	Assessment of Side Effects of AE F032640 00 EG06 A107 and AE F032640 00 EC03 B003 on the Honey bee (*Apis mellifera* L.) in the Semi-Field
Document No:	M-195280-01-1 (Rep. No.: 99379/01-BZEU)
Guidelines:	EPPO 170
GLP:	yes

~~**Material and methods**~~

~~The side effects of the two test substances AE F032640 00 EG06 A107 and AE F032640 00 EC03 B003 were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions according to the guideline of the European and Mediterranean Plant Protection Organization No. 170 (EPPO, 1992). The test substances, AE F032640 00 EG06 A107 and AE F032640 00 EC03 B003E, were applied at an application rate of 12.5 g a.s./ha in 300 L water/ha. Plots treated with tap water served as control. As toxic standard, Hostathion 40 EC was applied at a concentration of 0.6 L/ha in 300 L water/ha. The effect of the test substances was examined on small bee colonies in cages placed over plots with flowering *Phacelia tanacetifolia* Benth.~~

~~The influence of AE F032640 00 EG06 A107 and AE F032640 00 EC03 B003 was evaluated by comparing the effect of the two test substance variants to the effect of the control variant and toxic standard variant regarding the following observations:~~

- ~~Mortality at the edge of the treated area and in the bee traps~~
- ~~Foraging activity (number of forager bees/m² flowering *Phacelia* crop)~~
- ~~Behaviour of the bees on the crop and around the hive~~
- ~~Development of the bee brood~~

Findings:

Effect on honey bee mortality:

The application of both test substances AE F032640 00 EG06 A107 and AE F032640 00 EC03 B003 resulted in a slight increase of bee mortality restricted to the day of application (DAA 0).

During the pre-application period an average of 27.6 dead bees/colony/day was found in the AE F032640 00 EG06 A107 variant and 35.1 dead bees/colony/day in the AE F032640 00 EC03 B003 variant. The average daily pre-application level of mortality was 32.0 dead bees/colony/day in the control variant compared to 63.8 dead bees/colony/day in the toxic standard variant.

On the day of application mortality increased to an average of 97.7 dead bees/colony in the AE F032640 00 EG06 A107 variant. In the AE F032640 00 EC03 B003 variant an average of 139.0 dead bees/colony was found on the day of treatment.

A clear increase of mortality was observed after application of the toxic standard with a maximum of 549.3 dead bees/colony on the day of application.

When comparing the average pre-application mortality and the average post-application mortality utilizing $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality), a slight increase of mortality occurred in the two test substance variants and also in the control variant. An obvious increase was observed in the toxic standard variant. The value for $Q_{M(average)}$ was 1.4 in the AE F032640 00 EG06 A107 variant. In the AE F032640 00 EC03 B003 variant the $Q_{M(average)}$ was determined as 1.5. For the control variant and the toxic standard variant the $Q_{M(average)}$ value was 1.4 and 3.3, respectively.

Effects on honey bee flight intensity:

Shortly before application an average of 11.3 bees/m² was observed in the AE F032640 00 EG06 A107 variant and 13.0 bees/m² in the AE F032640 00 EC03 B003 variant. A clear decrease of flight intensity was observed after application of AE F032640 00 EG06 A107.

In the AE F032640 00 EG06 A107 variant an obvious repellent effect occurred directly after application and on evaluation day DAA 0 the flight intensity (5.9 bees/m²) remained clearly below the level of the control variant (13.4 bees/m²).

In the AE F032640 00 EC03 B003 variant the flight intensity remained on a high level (12.3 bees/m²) on the day of application.

On the following evaluation days flight intensities on the level of the control variant were observed in the two test substance variants. Compared with the pre-application period the average daily post-application level of flight intensity was higher in the two test substance variants and in the control variant but lower in the toxic standard variant.

Effects on honey bee brood development:

Regarding the colonies strength and the bee brood development, no abnormal differences attributable to the influence of the test substance were observed between the test substance variants and control.

Material and Methods:

Test material:	Deltamethrin EC 25 & EG 06
Test item:	Deltamethrin EG 6.25 (AE F032640 00 EG06 A107) content of a.s. (analysed): deltamethrin: 6.14 % w/w (62.5 g/L nominal) Deltamethrin EC 25 (AE F032640 00 EC03 B003) content of a.s. (analysed): deltamethrin: 2.70 % w/w (25 g/L nominal)
Batch number:	Deltamethrin EG 6.25: 8FES0248

	Deltamethrin EC 25: 99380
Reference item:	Hostathion 40 EC (active ingredient: triazophos; concentration of a.s.: 400 g a.s./L nominal, analysed content: not stated)
Test organism:	Honey bees (*Apis mellifera*) For the test, small healthy colonies with at least three combs (size of the combs ("Zandermaß"): 420 mm x 220 mm) were used. All nuclei were produced the same time. The corresponding queens originated from one breeding line in order to guarantee uniform bee material in all variants. Furthermore the following criteria for the nuclei were guaranteed: • at least two brood combs containing eggs, larvae and capped cells • at least one honey and pollen comb • bees are free of Nosema Wooden bee traps (35 cm x 35 cm) with gauze on bottom and on 50 % of the top were attached to the entrance of the nucleus in order to register those dead bees which are carried out of the hives.
Source:	Not stated in the report.
Crop:	*Phacelia tanacetifolia* (bee attractive crop) was full in bloom
Test location:	The semi-field test was located in the south of Germany [redacted]
Test unit:	The dimensions of the floor of the test cages were 4.8 m x 3.6 m and the height was 2 m. The cage frames were covered with light plastic gauze. The test cages were placed over the plots of flowering Phacelia. A path of 0.6 m was left at each side between the plots and the tent walls. The path was covered with linen sheet. Arrangement of the different variants during the semi-field test are shown in the following figure:

tent 12	tent 11	tent 10	tent 9	tent 8	tent 7	tent 6	tent 5	tent 4	tent 3	tent 2	tent 1
2T3	1T3	R3	C3	2T2	1T2	R2	C2	2T1	1T1	R1	C1

R = Toxic standard
1T = Test substance Deltamethrin EG 6.25
2T = Test substance Deltamethrin EC 25
C = Control

Application rates:	Control (C): drinking water Treatment rate (1T): Deltamethrin EG6.25: 12.5 g a.s./ha during foraging activity (≥ 10 bees/m^2 visiting flowers) Treatment rate (2T): Deltamethrin EC25: 12.5 g a.s./ha during foraging activity (≥ 10 bees/m^2 visiting flowers) Reference item (R): 0.6 L form./ha (240 g a.s./ha) Three replicates per treatment group referred to in this summary as: colony1, colony2 and colony3. The spray volume was 300 L/ha in all treatment groups.

Data analysis: The influence of the test substances Deltamethrin EG 6.25 and Deltamethrin EC 25 was evaluated by comparing the bees in the test cage to the control bees treated with water and those treated with the toxic standard and furthermore by comparing the pre- and post-application results in view of the following observations:

- Mortality at the edge of the treated area and in the bee traps
- Foraging activity (number of forager bees/m² flowering *Phacelia* crop)
- Behaviour of the bees on the crop and around the hive
- Development of the bee brood

Deviations from the study plan: Two deviations were recorded in the study report. As these were procedural deviations and do not impact the study results they have not been reported.

Climatic conditions during the experiment:

The environmental conditions are shown in the following table.

Table 1: Weather conditions during the trial; temperature and precipitation were provided by [redacted] weather station

Date	DAT	Ø Temperature min/max [°C]	Precipitation [mm]	Cloud formation at time of evaluation [%]
26/07/1999	- 2	12.8 / 27.7	0.0	0
27/07/1999	- 1	14.3 / 26.2	0.0	0
28/07/1999	0	16.4 / 27.0	0.0	0 - 20
29/07/1999	+ 1	12.1 / 27.0	0.0	0 - 10
30/07/1999	+ 2	11.1 / 27.0	0.0	0
31/07/1999	+ 3	10.9 / 27.3	0.0	0
01/08/1999	+ 4	12.3 / 28.2	0.0	0
02/08/1999	+ 5	11.5 / 28.6	0.0	0

Pesticide history of the field site:

The pesticide history of the field site was not stated in the report.

The side effects of the two test substances Deltamethrin EG 6.25 and Deltamethrin EC25 were tested on the honey bee (*Apis mellifera* L.) under semi-field conditions following the EPPO guideline No. 170 (EPPO, 1992).

This study included four exposure groups with three replicates (tunnels) each: one drinking water treated control group (C), two test-item groups (1T and 2T) and one reference item group (R). The hives were introduced into the test cages 2 days before the planned application of the test substances to enable the bees getting familiar with the environment and to lower the mortality which usually is increased due to the transport. In all exposure groups, the crop was sprayed at flowering stage of the crop, during which time honeybees were actively foraging on the crop under confined conditions. The honeybees remained 5 days in the tunnels following application.

The influence of the test substances Deltamethrin EG 6.25 and Deltamethrin EC25 was evaluated by comparing the bees in the test cage to the control bees treated with water and those treated with the toxic standard.

The following endpoints were assessed:

- Mortality at the edge of the treated area and in the bee traps at the entrance of the colonies before as well as after the application in the control, in the two treatments and reference item groups, respectively.
- Flight Intensity (number of bees that are both foraging on flowering Phacelia and flying immediately over the crop on 1 square meter) at the day the bee colonies set up into the tents and before as well as after the application in the control, in the two treatments and reference item groups, respectively.
- The condition of the colonies and the development of the bee brood were checked 2 days before application and 5 days after application. In order to record effects of the test substance, the following parameters were assessed:
  - Strength of the colony (number of combs covered with bees)
  - Presence of a healthy queen (presence of eggs, presence of queen cells)
  - Estimate of the pollen storage area and area with nectar
  - Estimate of the area containing eggs, larvae and capped cells

  The amount of eggs, larvae and capped brood was given in percent of total brood population for each type of brood.
- Behaviour of the bees on the crop and around the hive

Dates of Work: 26th July to 02nd August 1999

Findings:

Honey Bee Mortality

The application of both test items, Deltamethrin EG 6.25 and Deltamethrin EG 6.25, resulted in a slight increase of bee mortality restricted to the day of application (DAA 0aa).

During the pre-application period an average of 27.6 dead bees/colony/day was found in the Deltamethrin EG 6.25 variant and 35.1 dead bees/colony/day in the Deltamethrin EC 25 variant. The average daily pre-application level of mortality was 32.0 dead bees/colony/day in the control variant compared to 63.8 dead bees/colony/day in the toxic standard variant. On the day of application mortality increased to an average of 97.7 dead bees/colony in the Deltamethrin EG 6.25 variant. In the Deltamethrin EC 25 variant an average of 139.0 dead bees/colony was found on the day of treatment.

A clear increase of mortality was observed after application of the toxic standard with a maximum of 549.3 dead bees/colony on the day of application. When comparing the average pre-application mortality and the average post-application mortality utilizing $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality) a slight increase of mortality occurred in the two test substance variants and also in the control variant. An obvious increase was observed in the toxic standard variant. The value for $Q_{M(average)}$ was 1.4 in the Deltamethrin EG 6.25 variant. In the Deltamethrin EC 25 variant the $Q_{M(average)}$ was determined as 1.5. For the control variant and the toxic standard variant the $Q_{M(average)}$ value was 1.4 and 3.3, respectively.

A summary of the daily mortality and flight intensity results are shown in the following tables.

Table 2: Individual results of the evaluations of mortality (numbers of dead bees) in the Deltamethrin EG 6.25 variant at 12.5 g a.s./ha

Date	Day	Colony 1		Colony 2		Colony 3		Ø Colony and day
		BT	E	BT	E	BT	E	
26JUL99	2DBT	0	11	0	1	0	7	6.3
27JUL99	1DBT	1	9	1	17	2	81	37.0
28JUL99	0DBT	2	8	0	19	2	87	39.3
Ø pre-application		1.0	9.3	0.3	12.3	1.3	58.3	27.6
STD		1.0	1.5	0.6	9.9	1.2	44.6	18.4
28JUL99	0DAT	1	25	2	94	1	170	97.7
29JUL99	1DAT	0	9	0	9	0	28	15.3
30JUL99	2DAT	1	8	1	12	1	77	33.3
31JUL99	3DAT	0	15	1	22	0	45	27.7
01AUG99	4DAT	0	19	0	21	0	51	30.3
02AUG99	5DAT	1	25	0	14	0	50	30.0
Ø post-application		0.5	16.8	0.7	28.7	0.3	70.2	39.1
STD		0.5	7.5	0.8	32.4	0.5	51.4	29.4
Q_M		0.5	1.8	2.0	2.3	0.3	1.2	n.d.
$Q_{M(Oaa)}$		3.5						n.d.
$Q_{M(average)}$		1.4						n.d.

DBT = Days after treatment
DAT = Days after treatment
BT = Bee traps
E = Edge of the treated Phacelia area
STD = Standard deviation (calculation by Quattro Pro)
Oba = Mortality on the day of treatment before application
Oaa = Mortality on the day of treatment after application
Q_M = Post-application mortality / pre-application mortality
$Q_{M(Oaa)}$ = Ø Mortality on the day of application Oaa / Ø pre-application mortality
$Q_{M(average)}$ = Ø post-application mortality / Ø pre-application mortality

Table 3: Individual results of the evaluations of mortality (numbers of dead bees) in the Deltamethrin EC 25 variant at 12.5 g a.s./ha

Date	Day	Colony 1		Colony 2		Colony 3		Ø / Colony and day
		BT	E	BT	E	BT	E	
26JUL99	2DBT	0	9	1	2	1	7	6.7
27JUL99	1DBT	2	23	1	27	4	92	49.0
28JUL99	0DBT	0.7	41	1	14	2	89	49.7
Ø pre-application		1.2	24.3	1.0	14.3	2.3	62.7	35.1
STD		3	16.0	0.0	12.5	1.5	48.2	24.7
28JUL99	0DAT	0	74	6	68	3	263	139.0
29JUL99	1DAT	2	11	0	8	0	23	14.0
30JUL99	2DAT	2	32	0	18	0	45	31.7
31JUL99	3DAT	1	56	1	28	0	77	54.7
01AUG99	4DAT	3	53	0	13	0	64	43.7
02AUG99	5DAT	1.8	41	3	12	0	43	34.0
Ø post-application		1.2	44.5	1.7	24.5	0.5	85.5	52.8
STD		2.8	21.8	2.4	22.4	1.3	88.9	42.3
Q_M		0	1.8	1.7	1.7	0.2	1.4	n.d.
$Q_{M(Oaa)}$		4.0						n.d.
$Q_{M(average)}$		1.5						n.d.

DBT = Days after treatment
DAT = Days after treatment
BT = Bee traps
E = Edge of the treated Phacelia area
STD = Standard deviation (calculation by Quattro Pro)
Oba = Mortality on the day of treatment before application
Oaa = Mortality on the day of treatment after application
Q_M = Post-application mortality / pre-application mortality
$Q_{M(Oaa)}$ = Ø Mortality on the day of application Oaa / Ø pre-application mortality
$Q_{M(average)}$ = Ø post-application mortality / Ø pre-application mortality

Table 4: Individual results of the evaluations of mortality (numbers of dead bees) in the control variant .

Date	DAA	Colony 1		Colony 2		Colony 3		Ø / Colony and day
		BT	E	BT	E	BT	E	
26JUL99	2DBT	0	13	1	4	0	2	6.7
27JUL99	1DBT	2	44	1	54	2	43	48.7
28JUL99	0DBT	2	33	1	41	2	43	40.7
Ø pre-application		1.3	30.0	1.0	33.0	1.3	29.3	32.0
STD		1.2	15.7	0.0	25.9	1.2	23.7	22.3
28JUL99	0DAT	2	48	1	18	0	45	38.0
29JUL99	1DAT	0	11	0	9	0	7	9.0
30JUL99	2DAT	2	61	4	18	0	42	42.3
31JUL99	3DAT	0	81	3	35	2	65	62.0
01AUG99	4DAT	3	51	1	30	1	63	49.7
02AUG99	5DAT	0	82	9	39	1	56	62.3
Ø post-application		1.2	55.7	3.0	24.8	0.7	46.3	43.9
STD		1.3	26.2	3.3	11.6	0.8	21.4	19.8
Q_M		0.9	1.9	3.0	0.8	0.5	1.6	n.d.
$Q_{M(Oaa)}$		1.2						n.d.
$Q_{M(average)}$		1.4						n.d.

DBT = Days after treatment
DAT = Days after treatment
BT = Bee traps
E = Edge of the treated Phacelia area
STD = Standard deviation (calculation by Quattro Pro)
Oba = Mortality on the day of treatment before application
Oaa = Mortality on the day of treatment after application
Q_M = Post-application mortality / pre-application mortality
$Q_{M(Oaa)}$ = Ø Mortality on the day of application Oaa / Ø pre-application mortality
$Q_{M(average)}$ = Ø post-application mortality / Ø pre-application mortality

Honey Bee Flight Intensity

Shortly before application an average of 11.3 bees/m^2 was observed in the Deltamethrin EG 6.25 variant and 13.0 bees/m2 in the Deltamethrin EG 6.25 variant. A clear decrease of flight intensity was observed after application of Deltamethrin EG 6.25.

In the Deltamethrin EG 6.25 variant an obvious repellent effect occurred directly after application and on evaluation day DAA Oaa the flight intensity (5.9 bees/m2) remained clearly below the level of the control variant (13.4 bees/m2).

In the Deltamethrin EG 6.25 variant the flight intensity remained on a high level (12.3 bees/m^2) on the day of application.

On the following evaluation days flight intensities on the level of the control variant were observed in the two test substance variants. Compared with the pre-application period the average daily post-application level of flight intensity was higher in the two test substance variants and in the control variant but lower in the toxic standard variant.

Table 5: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the Deltamethrin EG 6.25 at 12.5 g a.s./ha (AE F032640 00 EG06 A107) group

Date	DAA	Ø Number of bees/m^2			Ø Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
26JUL99	-2	15.0	12.0	19.0	15.3
27JUL99	-1	16.0	14.0	19.0	16.3
28JUL99	0ba	13.0	11.0	10.0	11.3
Ø pre-application.		14.7	12.3	16.0	14.3
STD		1.5	1.5	5.2	2.6
28JUL99	0aa	6.5	4.8	6.3	5.9
29JUL99	1	20.3	12.3	21.7	18.1
30JUL99	2	16.0	14.0	24.0	18.0
31JUL99	3	21.0	12.0	22.0	18.3
01AUG99	4	24.0	28.0	17.0	26.3
02AUG99	5	24.0	25.0	13.0	20.7
Ø post-application.		20.3	16.0	17.3	17.9
STD		9.1	8.8	6.7	6.5

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application

Table 6: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the Deltamethrin EC 25 at 12.5 g a.s./ha (AE F032640 00 EC03 B003) group

Date	DAA	Ø Number of bees/m^2			Ø Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
26JUL99	-2	14.0	17.0	22.0	17.7
27JUL99	-1	12.0	16.0	15.0	14.3
28JUL99	0ba	13.0	14.0	12.0	13.0
Ø pre-application.		13.0	15.7	16.3	15.0
STD		1.0	1.5	5.1	2.4
28JUL99	0aa	10.5	15.5	10.8	12.6
29JUL99	1	14.0	22.3	21.7	19.3
30JUL99	2	11.0	27.0	16.0	18.0
31JUL99	3	18.0	18.0	22.0	19.3
01AUG99	4	22.0	38.0	22.0	27.3
02AUG99	5	12.0	26.0	28.0	22.0
Ø post-application.		14.6	24.5	20.1	19.7
STD		4.5	8.0	5.9	4.9

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application

Table 7: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the control group

Date	DAA	Ø Number of bees/m^2			Ø Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
26JUL99	-2	13.0	19.0	15.0	15.7
27JUL99	-1	14.0	12.0	11.0	12.3
28JUL99	0ba	12.0	14.0	15.0	13.7
Ø pre-application.		13.0	15.0	13.7	13.9
STD		1.0	3.6	2.1	1.4
28JUL99	0aa	13.5	12.8	13.8	13.4
29JUL99	1	17.0	20.7	22.7	20.1
30JUL99	2	13.0	20.0	25.0	19.3
31JUL99	3	19.0	24.0	17.0	20.0
01AUG99	4	17.0	30.0	30.0	25.7
02AUG99	5	18.0	18.0	22.0	19.3
Ø post-application.		16.3	20.9	21.8	19.6
STD		2.4	5.8	5.7	3.9

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application

Table 8: Average flight intensity (number of bees per m^2 Phacelia) in the three colonies in the toxic standard group

Date	DAA	Ø Number of bees/m^2			Ø Number of bees/m^2 and day
		Colony 1	Colony 2	Colony 3	
26JUL99	-2	20.0	18.0	16.0	18.0
27JUL99	-1	11.0	17.0	19.0	15.7
28JUL99	0ba	14.0	16.0	18.0	16.0
Ø pre-application.		15.0	17.0	17.7	16.6
STD		4.6	1.0	1.5	1.3
28JUL99	0aa	11.0	12.7	11.7	11.8
29JUL99	1	6.3	7.0	8.7	7.3
30JUL99	2	9.0	4.0	10.0	7.7
31JUL99	3	7.0	6.0	10.0	7.7
01AUG99	4	10.0	12.0	16.0	12.7
02AUG99	5	9.0	8.0	10.0	9.0
Ø post-application.		8.7	8.3	11.1	9.4
STD		1.8	3.4	2.6	2.3

DAA = Days after application
STD = Standard deviation (calculation by Quattro Pro)
Oba = Flight intensity on the day of treatment before application
Oaa = Flight intensity on the day of treatment after application

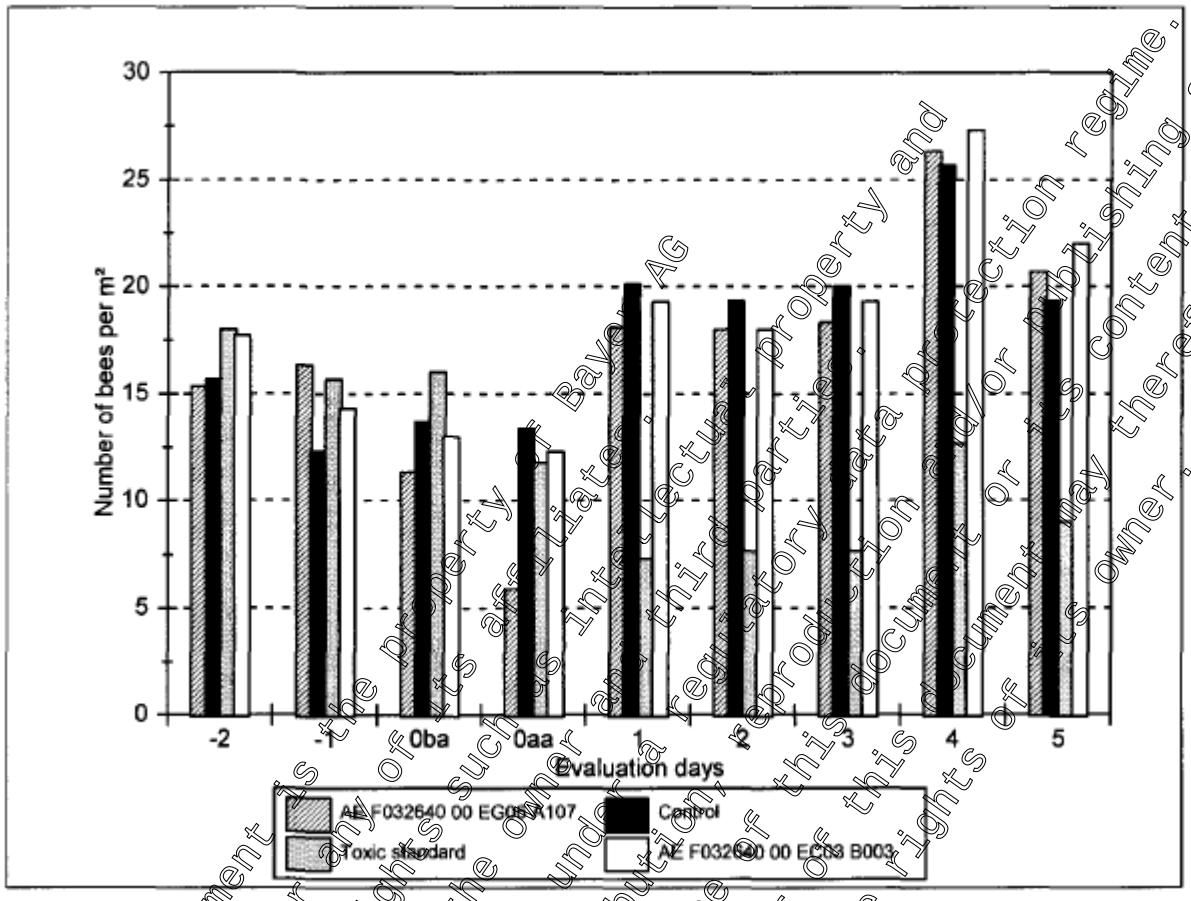


Figure 1: Average flight intensity in the test substance variants Deltamethrin EG 6.25 at 12.5 g a.s./ha (AE F032640 00 EG06 A107) and Deltamethrin EC 25 at 12.5 g a.s./ha (AE F032640 00 EC03 B003), control and toxic standard variant prior to and after application

Oba = evaluation on the day of treatment shortly before application

Oaa = evaluation on the day of treatment after application

Development of Honey Bee Brood

During the observation period changes and fluctuations in the relative amount of the different pre-imaginal stages, i.e. egg stage, larval and pupal stage, occurred in almost every colony of the test substance variants, control and toxic standard variant.

When the brood assessment was made 5 days after application, the area of brood and the strength of the colony was reduced in nearly all colonies.

The observed decrease must not be attributed to the influence of the test substances but can be expected, considering that the bees were kept in restricted spaces for 7 days and the limited food supply that would have been available within the small area of crop. The continued presence of eggs showed that the queens survived in all colonies.

Table 9: Brood development of the Deltamethrin EG 6.25 variant at 12.5 g a.s./ha

	Colony 1	Colony 2	Colony 3
Prior to application: 26JUL99			
Strength (No. of combs covered with bees)	2.0	3.5	2.0
No. of combs covered with brood	3	2	2
Average amount of egg stage in %	20.0	25.0	30.0
Average amount of larval stage in %	13.3	25.0	7.5
Average amount of capped stage in %	20.0	27.5	42.5
After application: 02AUG99			
Strength (No. of combs covered with bees)	2.0	2.5	2.5
No. of combs covered with brood	3	3	2
Average amount of egg stage in %	11.7	12.5	7.5
Average amount of larval stage in %	6.7	7.5	2.5
Average amount of capped stage in %	18.3	32.5	15.0

Table 10: Brood development of the Deltamethrin EC 25 variant at 12.5 g a.s./ha

	Colony 1	Colony 2	Colony 3
Prior to application: 26JUL99			
Strength (No. of combs covered with bees)	3.0	3.0	3.5
No. of combs covered with brood	3	3	2
Average amount of egg stage in %	18.3	16.7	12.5
Average amount of larval stage in %	8.3	16.7	17.5
Average amount of capped stage in %	21.7	18.3	65.0
After application: 02AUG99			
Strength (No. of combs covered with bees)	2.5	3.0	2.0
No. of combs covered with brood	3	3	2
Average amount of egg stage in %	13.3	6.7	12.5
Average amount of larval stage in %	13.3	3.3	0
Average amount of capped stage in %	18.3	21.7	37.5

Table 11: Brood development of the control variant

	Colony 1	Colony 2	Colony 3
Prior to application: 26JUL99			
Strength (No. of combs covered with bees)	2.5	2.5	2.0
No. of combs covered with brood	2	3	3
Average amount of egg stage in %	35.0	10.0	16.5
Average amount of larval stage in %	17.5	16.7	11.7
Average amount of capped stage in %	17.5	1.7	15.0
After application: 02AUG99			
Strength (No. of combs covered with bees)	2.5	2.5	2.0
No. of combs covered with brood	2	3	2
Average amount of egg stage in %	20.0	20.0	12.5
Average amount of larval stage in %	5.0	10.0	0
Average amount of capped stage in %	20.0	15.0	20.0

Table 12: Brood development of the toxic standard variant

	Colony 1	Colony 2	Colony 3
Prior to application: 26JUL99			
Strength (No. of combs covered with bees)	2.5	3.0	3.0
No. of combs covered with brood	2	2	2
Average amount of egg stage in %	20.0	30.0	20.0
Average amount of larval stage in %	12.5	20.0	15.0
Average amount of capped stage in %	25.0	22.5	65.0
After application: 02AUG99			
Strength (No. of combs covered with bees)	2.5	2.5	2.5
No. of combs covered with brood	2	2	2
Average amount of egg stage in %	20.0	20.0	10.0
Average amount of larval stage in %	20.0	5.0	5.0
Average amount of capped stage in %	20.0	17.5	42.5

Behaviour of the Bees

Directly after application of Deltamethrin EG 6.25 and Deltamethrin EG 6.25 the bees were observed rising up of the flowering Phacelia, but only in the Deltamethrin EG 6.25 variant the flight intensity remained on a low level on the evaluation day DAA Oaa.

Conclusion:

The application of both test substances, Deltamethrin EG 6.25 and Deltamethrin EG 6.25, resulted in a

slight increase in honey bee mortality restricted to the day of application (DAA Oaa). An average 97.7 dead bees/colony was found on the day of treatment in the test substance variant Deltamethrin EG 6.25 and 139.0 dead bees/colony in the test substance variant Deltamethrin EG 6.25 compared to 38.0 dead bees/colony in the control variant. A clear increase of mortality was observed after application of the toxic standard with a maximum of 549.3 dead bees/colony on the day of application. The effect of the toxic standard demonstrates the sensitivity of the method in detecting the toxic effects of a pesticide. When comparing the average pre-application mortality and the average post-application mortality utilizing $Q_{M(average)}$ (average post-application mortality divided by the average pre-application mortality) a slight increase of mortality occurred in the two test substance variants and also in the control variant. An obvious increase was observed in the toxic standard variant. The value for $Q_{M(average)}$ was 1.4 in the Deltamethrin EG 6.25 variant. In the Deltamethrin EG 6.25 variant $Q_{M(average)}$ was determined as 1.5. For the control variant and the toxic standard variant the $Q_{M(average)}$ value was 1.4 and 3.3, respectively.

In the Deltamethrin EG 6.25 variant an obvious repellent effect occurred directly after application and on this day the flight intensity (5.9 bees/m²) remained clearly below the level of the control variant (43.4 bees/m²). Deltamethrin EG 6.25 variant the flight intensity remained on a high level (12.3 bees/m²) on the day of application. On the following evaluation days flight intensities on the level of the control variant were observed in the two test substance variants.

Regarding the colonies strength and the bee brood development no abnormal differences attributable to the influence of the test substance were observed between the test substance variants and the control.

*****

Report:	**KCP 10.3.1.5/11, [redacted]; 1999**
Title:	Impact of Decis micro and Decis EC on honey bees - insectproof tunnels on phacelia crop
Document No:	M-195036-01-1 (Rep. No.: 999)
Guidelines:	CEB 129
GLP:	yes

~~Material and Methods:~~

~~Honey bee colonies (ca 20,000 bees per hive, colonies of comparable development, brood, and food store status, queens of homogeneous maternal origin) were confined in tunnels on *Phacelia* fields with additional pollen sources provided. Five days after introduction of the bees into the tunnels, application was performed. The test substances Decis micro and Decis EC were both applied at a rate corresponding to 12.5 g a.s./ha. Furthermore, a toxic reference and a water-treated control was set up. Treatment was carried out during flight activity of the bees. Endpoints observed were foraging activity, behavior, mortality, and colony development.~~

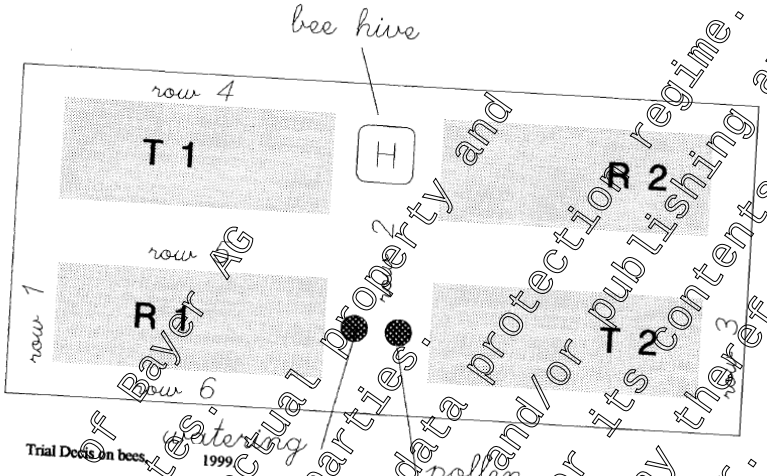
~~Findings:~~

~~The application of Decis EC, which only slightly disturbed foraging, resulted in a mortality peak the day after treatment, thereafter, daily mortalities were comparable to control. The treatment with Decis EC did not have any further effects. Decis micro showed intermediairy effects, with moderate impact on mortality and restricted to the day after product application.~~

~~Apiarist visits carried out during the trial allow us to think that all colonies were sufficiently viable to ensure their further development. After the trial, all hives have been returned to professional beekeepers who will put them into use again.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltemethrin EG 6.25 (Décis micro, AE F032640 00 EG06 A107, 6.25 %, 62.5 g/kg nominal, analysed content not stated in the report) Deltemethrin EC 25 (Décis EC, AE F032640 00 EC03 B008, 25 g a.s./L nominal)
Batch number:	Décis micro: 8FES0248 Décis EC: 7CD11324
Reference item:	Zolone Flo (active ingredient: phosalone, 500 g a.s./L nominal)
Test organism:	Honey bees (*Apis mellifera*) Bee colonies came from the same apiary containing 1,350 hives allowing easy selection of swarms. Among the hives considered, seven were chosen according to their homogeneity during the weeks preceding the test and three of the hives were introduced in the tunnels. Young honey bee colonies with queens from the local black breed which were one year old. The queens had a common genetic identity, they were sisters (or half-sisters) coming from a single strain. The colonies lived in hives of the DADANT 12 frames model readjusted to proportions of DADANT 10 frames through a feeder frame placed inside on one of the sides. Populations spread over 9 to 10 frames (of which approximately 2 to 4 frames of brood) have been estimated at around 20,000 bees per hive.
Source:	Not stated in the report.
Crop:	Phacelia crop of the Phaci variety at flowering stage.
Test location:	[redacted] on a field from [redacted] France
Test unit:	Each tunnel had a half-moon support made from galvanized steel; the hoops were nailed in the soil and joined with crossbars. The surface per unit was 140 m² (7 m x 20 m) and their roofheight approximately 3 metres. A polyethylene mesh net (1.2 mm x 1.2 mm) covered the supports. Both ends were made up of the same material. Access was possible through a zip opening. Inside the tunnels, the Phacelia crop was split into four plots. Each had a surface of 16 m² (2m x 8m), two plots were considered as sheltered areas (R1 and R2, not treated with test item), the other two (T1 and T2) as treated areas. A beehive, a watering place and feeders with pollen were placed in each of the tunnels and supplied daily. Exact interior design of the tunnel is shown in the figure below:



R: sheltered area
T: treated area

Application rates: Control (C): water
Deltemethrin EC 6.25: 12.5 g a.s./ ha during foraging activity
Deltemethrin EC 25: 12.5 g a.s./ ha during foraging activity
Reference rate (R): 1.2 L/ ha (500 g a.s./L)
Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).
The spray volume was 300-315 L/ha in all treatment groups. The sprayer was calibrated before use.

Data sampling: Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.

Data analysis: All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.

Deviations from the study plan: No deviation mentioned

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperature favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table.

Table 1: Weather data

	minimum Temperature (°C)	maximum Temperature (°C)	Rainfall (mm)
4 September 99	8	28	0
5 September 99	12	29	0
6 September 99	14	27	0
7 September 99	12	24	0
8 September 99	9	28	0
9 September 99	9	26	0
10 September 99	12	28	0
11September 99	7	29	0
12 September 99	12	20	0
13 September 99	10	12	27
14 September 99	9	12	10
15 September 99	3	16	0

N.B. : Temperatures are given for the day mentioned, whereas rainfall recorded every morning shows precipitation over the previous 24 hours.

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 2: *Phacelia* crop data

Date	Operation	Caracteristics
May / June	Soil preparation	Herbicide application and harrowing : seedbed preparation and weed destruction
25/06/99	4th plot drilling	PHACI variety at 7.5 kg per ha
25-28/06/99	Maintenance	Rolling and watering the plot
22/09/99	Destruction	Crushing the crop on experimental plots

The effects of Deltemethrin EG 6.25 (Decis micro) and Deltemethrin EC 25 (Decis EC) were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the guidance document C.E.B. method no. 129. The aim of the study was to evaluate potential side effects of a spray application of Deltemethrin EG 6.25 and Deltemethrin EC 25 on the honeybee, *Apis mellifera* under forced exposure conditions.

This study included four exposure groups (tunnels) with one replicate each: one water treated control group, two test-item groups, one with Deltemethrin EC 25 and one with Deltemethrin EG 6.25 and one reference item group.

The hives were introduced into the tunnels five days prior to product application, in order to await a mortality decrease and stabilisation. The colonies were comparable to each other during our first visit at

the beginning of the test period, and mortality was homogeneous the first day of the study. Mortality in each tunnel was recorded on a daily basis for all areas covered with plastic film, from days 4DBT to 6DAT. Moreover, the day on which product application was carried out (day 0) additional counts were done at the end of the day (0DAT) in order to establish possible brutal intoxication of foraging bees. The total mortality rate recorded in a tunnel for a given day results from adding up mortality rates observed in each of the six plasticised rows in the tunnel.

Foraging was observed three to four times per day, whenever possible, on all treated (T1 + T2) and sheltered (untreated, R1 + R2) areas. It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts could be shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall...etc.) This parameter was also taken into account for an additional count on the day of treatment (day 0), during the hour following product application.

Two colony assessments were carried out in the beginning and at the end of experimentation allowing to evaluate colony development taking into account parameters like the adult bee population, the quantity and quality of the brood (different stages observed), amount of reserves and potential construction of new frames on offered wax sheets. These visits were carried out in the tunnels at dates which were as close as possible to the first and last day of confinement. However, for practical or climatic conditions, they necessarily took place within 48 hours before or after introduction of the hives in the tunnels on the one hand, and when the hives were taken out on the other hand.

Assessments of bee behaviour were carried out when products were applied and during the thirty minutes following product application. In general, this observation phase continued all over the day, between counts. Bees were especially observed for reactions and behaviour like intense flying, bee clusters on the net or at the entrance of the hive, aggressiveness, beginning of an intoxication etc. in each of the tunnels.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item group and the reference item group, respectively
- Number of foraging bees/m² per day on all the areas (T1, T2 and R1, R2) before as well as after the applications in the control, the test item group and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the test item groups and the reference item group, respectively
- Colony Assessment in the beginning and at the end of experimentation

Dates of Work: 5th July to 16th September 1999

Findings:

Honey bee mortality

A summary of the daily mortality and total mortality results are shown in the following table.

Table 3: Daily mortality data

Treatment	4DBT - 06 September						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	115	60	147	63	11	75	**471**
Water control	145	89	276	164	4	131	**809**
Deltemethrin EG 6.25 at 12.5 a.s./ha	59	72	84	36	4	29	**284**
Deltemethrin EC 25 at 12.5 a.s./ha	89	51	153	42	3	51	**389**
Treatment	**3DBT - 07 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	106	28	31	21	7	23	**216**
Water control	279	27	24	40	3	44	**417**
Deltemethrin EG 6.25 at 12.5 a.s./ha	166	22	65	34	3	48	**338**
Deltemethrin EC 25 at 12.5 a.s./ha	181	19	25	16	4	45	**290**
Treatment	**2DBT - 08 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	78	23	31	16	7	21	**176**
Water control	269	33	19	40	4	39	**404**
Deltemethrin EG 6.25 at 12.5 a.s./ha	206	19	62	20	4	60	**371**
Deltemethrin EC 25 at 12.5 a.s./ha	201	20	55	19	4	60	**359**
Treatment	**1DBT - 09 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	58	38	39	25	4	40	**204**
Water control	142	22	84	51	3	49	**351**
Deltemethrin EG 6.25 at 12.5 a.s./ha	63	49	44	23	2	31	**212**
Deltemethrin EC 25 at 12.5 a.s./ha	120	32	114	24	6	53	**349**
Treatment	**0DBT morning - 10 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	71	52	33	43	3	27	**229**
Water control	129	28	41	89	2	24	**313**
Deltemethrin EG 6.25 at 12.5 a.s./ha	62	59	24	37	6	15	**203**
Deltemethrin EC 25 at 12.5 a.s./ha	88	44	60	48	1	32	**273**
Treatment	**0DAT evening - 10 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	39	13	19	23	4	23	**121**
Water control	64	23	63	71	8	28	**257**
Deltemethrin EG 6.25 at 12.5 a.s./ha	88	97	113	145	6	19	**468**
Deltemethrin EC 25 at 12.5 a.s./ha	108	40	332	131	5	63	**679**

Treatment	1DAT - 11 September						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	21	22	15	18	4	24	**104**
Water control	50	12	39	27	6	14	**148**
Deltemethrin EG 6.25 at 12.5 a.s./ha	62	49	88	49	8	8	**264**
Deltemethrin EC 25 at 12.5 a.s./ha	98	22	169	80	10	37	**416**
Treatment	**2DAT – 12 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	49	28	34	25	3	24	**163**
Water control	119	30	39	40	7	42	**277**
Deltemethrin EG 6.25 at 12.5 a.s./ha	73	66	36	34	3	41	**253**
Deltemethrin EC 25 at 12.5 a.s./ha	62	27	40	32	4	43	**208**
Treatment	**3DAT – 13 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	85	20	16	30	4	41	**196**
Water control	294	32	27	25	13	50	**441**
Deltemethrin EG 6.25 at 12.5 a.s./ha	114	123	20	52	20	47	**376**
Deltemethrin EC 25 at 12.5 a.s./ha	123	42	24	43	16	85	**333**
Treatment	**4DAT – 14 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	31	26	4	4	2	9	**79**
Water control	78	17	12	5	2	12	**126**
Deltemethrin EG 6.25 at 12.5 a.s./ha	33	49	5	22	2	14	**115**
Deltemethrin EC 25 at 12.5 a.s./ha	40	27	8	14	4	27	**120**
Treatment	**5DAT – 15 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	36	15	2	11	1	7	**72**
Water control	79	5	1	13	2	13	**113**
Deltemethrin EG 6.25 at 12.5 a.s./ha	40	40	1	16	4	2	**103**
Deltemethrin EC 25 at 12.5 a.s./ha	68	18	1	6	2	21	**116**
Treatment	**6DAT – 16 September**						
area	row 1	row 2	row 3	row 4	row 5	row 6	total
Zolone Flo	64	29	6	14	3	11	**127**
Water control	83	32	11	39	3	13	**181**
Deltemethrin EG 6.25 at 12.5 a.s./ha	98	57	19	35	12	11	**232**
Deltemethrin EC 25 at 12.5 a.s./ha	129	34	27	22	6	17	**235**

DBT: days before treatment
DAT: days after treatment

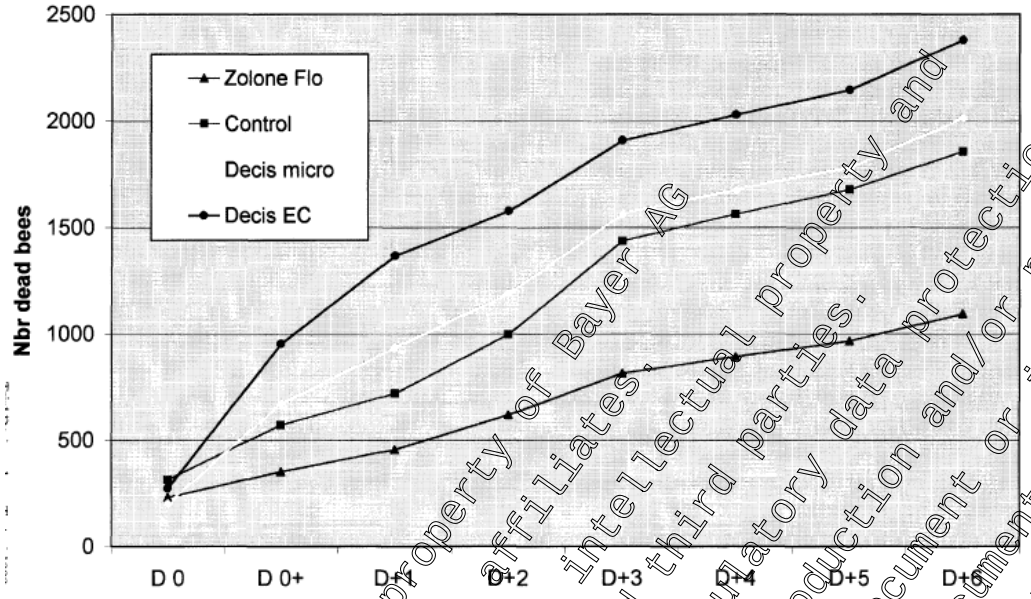


Figure 1: Total mortalities
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+6: 1 to 7 days after treatment

After 5 days of confinement, mortality rates were comparable for the six tunnels.

The day after the treatment, mortality trends showed quite some differences. Only the Deltemethrin EC 25 formulation showed a very distinct mortality peak at 1DAT. This phenomenon was very spectacular but also very brief. From 2DAT the mortality rates recorded in this tunnel litterally dropped to a level which was lower than the one from the pre-treatment phase and remained very low until the end of the tests.

On the other hand, the standard tunnel treated with phosalone showed the least variation in daily mortality, maybe because this colony was less active. In the control tunnel treated with water the colony was stronger, but it was not disturbed by a water treatment. Mortality rates recorded varied because the colony's activity was ruled by climatic conditions. This variation was limited and gives an indication of mortality rates in other tunnels.

The speciality Deltemethrin EG 6.25 showed an increase in intermediary mortality rate the day after product application between that of the control and Deltemethrin EC 25. This increase mortality was not very high, and it is remarkable that levels of mortality at 2DAT showed the same order of magnitude in the four tunnels.

The graph confirms that mortality rate trends are similar in the two tunnels: Deltemethrin EG 6.25 and the water control. Daily mortality rates evolve in the same way and only start showing differences from 1DAT onwards. No spraying effects were observed in the standard tunnel, but they were obvious in the Deltemethrin EC 25 tunnel.

Distribution of dead bees within the tunnels did not give any additional information. This distribution was mainly influenced by wind and maybe by the sunlight guiding the bees, because most of the data were recorded in the rows at both ends of the tunnels (A1 or A3).

Foraging activity

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following tables.

Table 4: Foraging data: Zolone Flo

tunnel 1		Number of bees per observation area												nb bees /m²		
day	time	R1a	R1b	R1t	T1a	T1b	T1t	R2a	R2b	R2t	T2a	T2b	T2t	R	T	mean zt
07 Sept 99	12H00	71	74	145	73	53	126	54	43	97	51	58	109	8	7	118
D-3	15H00	76	94	170	83	61	144	77	40	117	59	66	125	9	8	135
	16H45	93	100	193	85	86	171	65	46	111	56	77	133	10	10	152
																135
08 Sept 99	12H00	91	73	164	92	103	195	44	46	90	57	71	128	8	10	162
D-2	15H30	98	108	206	119	119	238	79	57	136	67	76	143	11	12	191
	16H30	111	112	223	107	118	225	77	57	134	81	99	180	11	13	203
																185
09 sept 99	11H00	83	67	150	88	71	159	58	49	107	52	45	97	8	8	128
D-1	12H00	86	85	171	99	61	160	68	41	109	75	56	131	9	9	146
	14H30	85	104	189	142	80	222	61	47	108	69	61	130	9	11	178
																150
10 sept 99	11H00	102	77	179	93	139	232	47	54	101	63	69	132	9	11	182
D 0	13H15	82	84	166	95	112	207	56	56	112	61	74	135	9	11	171
																177
	15H00	96	77	173	61	85	146	34	59	93	64	64	128	8	5	132
	16H00	84	89	173	124	108	232	46	55	100	57	63	120	9	11	178
	17H00	82	92	174	96	112	208	47	50	97	79	70	149	8	11	179
																162
11 sept 99	12H00	79	75	154	114	88	202	69	46	115	86	63	149	8	11	176
D+1	15H00	72	102	174	119	102	221	81	63	144	77	78	155	10	12	188
	16H30	81	114	195	128	90	218	92	62	154	78	77	155	11	12	197
																183
12 sept 99	11H00	11	11	22	12	17	29	8	8	16	8	5	13	1	1	20
D+2	13H30	65	66	131	76	96	172	54	62	116	59	62	121	8	9	147
	14H30	63	68	131	108	79	187	59	51	110	57	60	117	8	10	152
	15H30	69	54	123	88	83	171	62	39	101	54	44	98	7	8	135
																144

Counts carried out on 12 Sept. at 11 am, not significant, are not taken into account in our calculation.

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b: number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m^2 : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] / 16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m^2.
D-3 to D-1: days before treatment
D0: day of treatment
D+2/ D+2: 1/ 2 days after treatment

Table 5: Foraging data: Water control

		Number of bees per observation area												nb bees./m²		
day	time	R1a	R1b	R1t	T1a	T1b	T1t	R2a	R2b	R2t	T2a	T2b	T2t	R	T	mean zt
07 sept 99	12H00	94	103	197	115	120	235	41	29	70	57	76	133	8	12	184
D-3	15H00	108	148	256	108	127	235	40	36	76	66	94	160	10	12	198
	16H45	109	164	273	126	162	288	45	65	110	60	101	161	12	14	225
																202
08 sept 99	12H00	94	149	243	112	116	228	34	48	82	48	98	146	10	12	187
D-2	15H30	129	173	302	145	151	296	59	73	132	74	111	185	14	15	241
	16H30	117	191	308	143	148	291	72	77	149	68	139	207	14	16	249
																226
09 sept 99	11H00	90	90	180	104	104	208	44	42	86	62	58	120	8	10	184
D-1	12H00	91	109	200	139	118	257	45	49	94	64	91	155	9	13	206
	14H30	137	138	275	163	146	309	63	57	120	62	107	169	12	15	239
																203
10 sept 99	11H00	106	109	215	102	133	235	36	47	83	74	78	152	9	12	194
D 0	13H15	121	127	248	154	137	291	43	46	89	83	95	178	11	15	235
																214
	15H00	112	133	245	136	97	233	40	59	99	99	99	198	11	12	196
	16H00	126	83	209	162	118	280	41	68	109	73	102	175	10	14	228
	17H00	111	106	217	157	139	296	50	48	98	80	102	182	10	15	239
																221
11 sept 99	12H00	116	126	242	119	174	293	66	65	131	76	106	182	12	15	238
D+1	15H00	121	114	235	132	176	308	59	54	113	88	86	174	11	15	241
	16H30	110	121	231	153	151	304	64	55	119	89	117	206	11	16	255
																245
12 sept 99	11H00	19	19	38	28	16	44	5	6	11	7	9	16	2	2	30
D+2	13H30	79	104	183	85	111	196	43	49	92	55	73	128	9	10	162
	14H30	107	82	189	112	122	234	60	32	92	76	91	164	9	12	199
	15H30	93	90	183	115	83	198	54	34	88	57	78	135	8	10	167
																176

Counts carried out on 12 Sept. at 11 am, not significant, are not taken into account in our calculation.

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m²: number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16 Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-3 to D-1: days before treatment
D0: day of treatment
D+2/ D+2: 1/ 2 days after treatment

Table 6: Foraging data: Deltemethrin EG 6.25 (Decis micro) at 12.5 a.s./ha

		Number of bees per observation area												nb bees/m²		
day	time	R1a	R1b	R1t	T1a	T1b	T1t	R2a	R2b	R2t	T2a	T2b	T2t	R	T	mean zt
07 sept 99	12H00	127	154	281	58	60	118	80	84	164	109	117	226	14	11	172
D-3	15H00	151	179	330	64	118	182	89	103	192	127	129	256	16	14	219
	16H45	183	181	364	105	142	247	124	140	264	166	167	333	20	18	290
																227
08 sept 99	12H00	149	171	320	95	105	200	115	130	245	140	190	330	18	17	265
D-2	15H30	156	179	335	100	166	266	162	152	314	187	188	375	20	20	321
	16H30	159	201	360	114	172	286	144	180	324	178	198	376	21	21	331
																306
09 sept 99	11H00	137	108	245	128	89	217	137	106	243	149	122	271	16	15	244
D-1	12H00	156	143	299	123	107	230	139	135	274	141	162	303	18	17	267
	14H30	205	162	367	165	133	298	192	176	368	223	180	403	23	22	351
																287
10 sept 99	11H00	119	105	224	99	112	211	102	105	207	101	122	223	13	14	217
D 0	13H15	162	139	301	143	126	269	136	118	254	153	131	284	17	17	277
																247
	15H00	184	121	305	67	89	156	68	152	220	128	103	231	16	12	194
	16H00	173	139	312	74	99	173	86	122	208	131	127	258	16	13	216
	17H00	195	111	306	70	110	180	96	114	210	142	144	286	16	15	238
																216
11 sept 99	12H00	144	152	296	97	125	222	101	140	241	151	137	288	17	16	255
D+1	15H00	165	175	340	106	133	239	118	146	264	139	171	310	19	17	275
	16H30	174	182	356	137	155	292	131	137	268	177	186	363	20	20	328
																286
12 sept 99	11H00	12	14	26	6	8	14	9	7	16	14	15	29	1		22
D+2	13H30	118	116	234	108	103	211	85	92	177	114	104	218	13	13	215
	14H30	141	129	270	124	127	251	130	153	283	121	114	235	17	15	243
	15H30	123	99	222	101	85	186	99	84	183	119	83	202	13	12	194
																217

Counts carried out on 12 Sept. at 11 am, not significant, are not taken into account in our calculation.

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] / 16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².
D-3 to D-1: days before treatment
D0: day of treatment
D+2/ D+2: 1/ 2 days after treatment

Table 7: Foraging data: Deltamethrin EC 25 (Decis EC) at 12.5 a.s./ha

		Number of bees per observation area												nb bees/m²		
day	time	R1a	R1b	R1t	T1a	T1b	T1t	R2a	R2b	R2t	T2a	T2b	T2t	R	T	mean zt
07 sept 99	12H00	161	140	301	137	157	294	127	122	249	145	122	267	17	18	281
D-3	15H00	161	130	291	145	153	298	162	126	288	142	147	289	18	18	294
	16H45	164	149	313	162	169	331	156	120	276	138	138	276	18	19	304
																293
08 sept 99	12H00	137	175	312	147	127	274	163	158	321	154	163	317	20	18	296
D-2	15H30	177	180	357	179	192	371	193	183	376	187	186	373	23	20	372
	16H30	161	177	338	152	202	354	184	186	370	187	181	368	22	23	361
																343
09 sept 99	11H00	133	123	256	176	150	326	177	149	326	172	115	287	18	19	307
D-1	12H00	147	146	293	175	152	327	181	132	313	183	141	324	19	20	386
	14H30	199	142	341	181	148	329	186	166	352	214	162	376	22	22	359
																328
10 sept 99	11H00	115	94	209	129	129	258	112	101	213	125	79	204	13	14	231
D 0	13H15	133	156	289	163	172	335	171	149	320	191	112	303	19	20	319
																275
	15H00	140	139	279	151	133	284	147	152	299	114	117	231	18	16	258
	16H00	138	144	282	144	170	314	155	159	314	142	121	263	19	18	289
	17H00	161	135	296	107	138	245	161	153	314	121	127	248	19	15	247
																264
11 sept 99	12H00	112	159	271	127	137	264	145	109	254	158	108	266	16	17	265
D+1	15H00	129	144	273	138	162	300	149	173	322	151	169	320	19	19	310
	16H30	132	174	306	145	163	308	165	182	347	160	140	300	20	19	304
																293
12 sept 99	11H00	17	19	36	25	15	40	25	12	37	19	9	28	2	2	34
D+2	13H30	105	80	185	127	126	253	118	122	240	106	103	209	13	14	231
	14H30	102	138	240	133	123	256	124	131	255	126	137	263	15	16	260
	15H30	100	93	193	105	147	252	112	138	250	118	142	260	14	16	256
																249

Counts carried out on 12 Sept. at 11 am, not significant, are not taken into account in our calculation.

R1a: number of bees counted on half of the sheltered area n°1 by a first experimentator.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimentator.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimentator.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimentator
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m2 : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2 D-3 to D-1: days before treatment
D0: day of treatment
D+2/ D+2: 1/ 2 days after treatment
] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m²
D-3 to D-1: days before treatment
D0: day of treatment
D+2/ D+2: 1/ 2 days after treatment

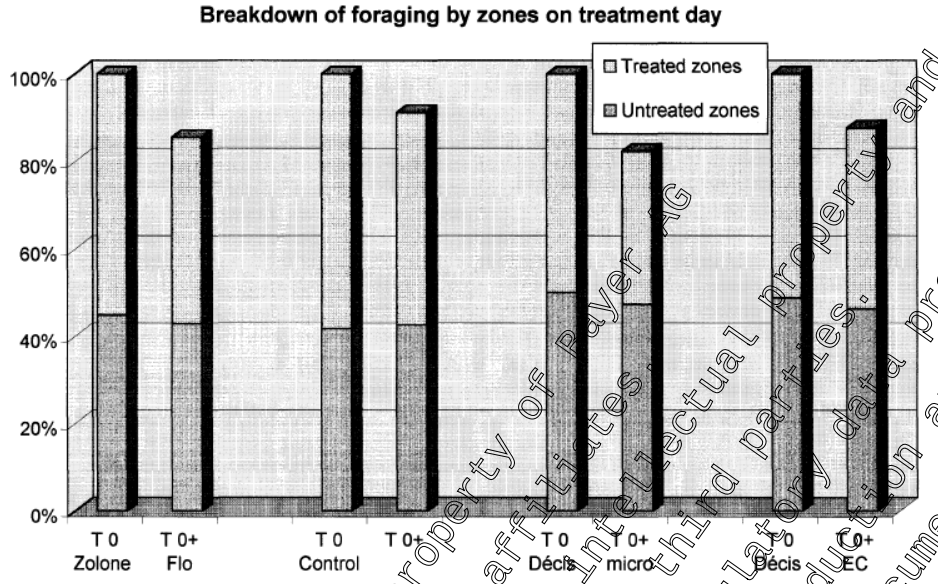


Figure 2: Breakdown of foraging on treatment day for Deltemethrin EC 25 (Decis EC) at 12.5 a.s./ha, Deltemethrin EG 6.25 (Decis micro) at 12.5 a.s./ha, water control and the reference item (Zolone Flo)

T0: before product application

T0+: after product application

At the beginning of the experimental phase, following introduction of the bee hives in the tunnels, bees foraged floral buds quite actively. Mean daily thresholds of 8 to 20 bees per m^2 were reached during the three days before product application.

During the three counts that followed product application, mean foraging trends were different between tunnels. Foragers' activity was slowed down following product application in all tunnels where test products or standard products were used.

In the control tunnel, where water spraying did not disturb the foragers' activity, on the contrary, it slightly increased during the day.

Following products applications, foraging activity evolved comparatively between Deltemethrin EG 6.25 tunnel, Deltemethrin EC 25 and reference tunnel. These three specialities disturbed foraging, at least temporary but didn't stop it. In fact this activity slowed down but stayed at comparable levels than earlier.

In all tunnels, foraging thresholds remained quite high the day after product application (1DAT), at comparable levels to those obtained over several days, always between 11 and 20 bees per m^2 on average, with even higher activity peaks during the day in the most favourable conditions.

On 2DAT, acceptable overall weather conditions during the day were beneficial to the bees, but their activity started to reduce in the six tunnels in spite of the colonies' vitality when clouds and temperature started to degrade (rainy storm on 3DAT).

After product application (0DAT, during the thirty minutes following product application), none of the tunnels showed increased activity, this explains levels under 100 %. It is, however, preferable to talk about relative foraging stability in most of the tunnels because differences are not significant.

Colony Assessment

There was little difference concerning the structure of the colonies between the two visits. The state of the reserves and proportions of the brood remained stable, but nectar flow from the Phacelia caused blocking in egg production. After eleven days confinement this colony had no brood, neither open or capped, because foragers had concentrated on storing reserves (+ 1 or 2 frames). This phenomenon could also be observed with other colonies in the control and Deltemethrin EG 6.25 tunnels. These colonies had frames with capped brood, but no more uncapped brood or eggs, implying probable interruption of egg-laying by the queens.

All the frames had been built in the beginning of the tests, there were only a few wax bees and their main role was to maintain the wax cells. In the other hives the queens continued laying eggs, but populations did not grow. The very significant foraging activity, however should have encouraged colony growth. Reserves only increased slightly during the tunnel confinement phase, so most of the activity was justified by the needs for food of this colony. The high foraging activities observed on experimental plots depended on the size of the swarms contained by those hives.

Even though bees died every day, their deaths were compensated by the emergence of new bees on the brood frames, and populations decreased only little during the test.

Behaviour of the bees

Colony behaviour was comparable between tunnels, as foraging was quite regular on Phacelia plots. Colonies in the different tunnels only showed little reaction to treatments, if it were not for flying away when the boom with water passed by.

In the standard tunnel, a characteristic Zolone smell appeared after treatment and remained for several hours. A few intoxication signs also appeared and were more frequent by the end of the day, but did not have any consequences on the next day (1DAT).

Activity at the hive entrance was normal in all six tunnels. No bee clusters were observed on the nets nor at the hive entrance and no fleeing events were observed in any of the tunnels.

Intoxication symptoms: description had to be compared to what happened in the standard tunnel. Foragers in contact with the product were the ones that were affected first. In Deltemethrin EG 6.25 and Deltemethrin EC 25 tunnels, some bees were on the ground after treatment and they had typical intoxication signs, similar to phosalone.

In these tunnels, intoxicated bees fell on the plastic surface of the rows, walked in a difficult way alternating periods of sluggishness and frenzy. Such a bee rolled over itself and appeared too heavy when trying to lift off. Its fore legs then its hind legs and abdomen appeared to be paralysed. The bee died in a range from a few minutes to a few hours.

Conclusion:

Overall conditions for conducting this experimental phase of the scheme were favourable to bee activity. Climatic and crop conditions were satisfactory. The different parameters observed agreed with the results obtained.

On this trial, Deltemethrin EC 25, which only slightly disturbed foraging, yielded a strong mortality increase, which was characterised by a very clear peak at 1DAT. It did not have any further effects.

Deltemethrin EG 6.25 showed intermediary effects, with moderate impact on mortality and restricted to the day after product application.

Under the experimental conditions of this study, the phosalone standard behaved similarly to the control and only had a limited effect on the behaviour and the development of the bee colony.

Based on apiarist visits carried out during the trial the viability of colonies was considered sufficient for their further development.

Experimental conditions of the study were quite strict, including confinement and product application carried out during intense foraging activity, on very attractive plots. Only the use of Deltemethrin EC 25 gave a high mortality peak the day after treatment and then daily mortalities were comparable. The effects of the other trial substance, Deltemethrin EG 6.25, only showed a temporary increase in mortality yielding comparable total mortality rates to those recorded in the control tunnel.

*****

Report:	**KCP 10.3.1.5/12, [redacted]: 2001**
Title:	Tunnel test - Acute and short term effects of AE F032640 00 EW01 B106, applied on cereals, on honey bees (*Apis mellifera* L.)
Document No:	M-203985-01-1 (Rep. No.: S01AVB879VO45)
Guidelines:	EPPO 170, (1992), CEB 129
GLP:	yes

~~**Material and Methods:**~~
~~Bees were confined within tunnels on winter wheat fields (cereals sprayed with sugar solution in order to provide food resources to the bees). After an acclimatization phase of four days, application was performed during bee flight. The control was treated with water, the test item was applied at a rate of 0.417 L/ha, as a non-toxic standard, Zolone Flo was used at a rate of 1.2 L/ha. The test substance treatment was twice replicated, control and standard once. Endpoints assessed were mortality, foraging and flight activity, storage of honey and pollen, behavior, and brood development.~~

~~**Findings:**~~
~~Mortality and flight activity in the test substance treatment were similar as in the non-toxic standard. Foraging activity was not or only very slightly affected by the test substance treatment on the treated as well as on the refuge areas in the tunnels. Likewise, no effects on the behavior were detected. Brood development was not affected by the test substance treatment as well.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EW 15 (Decis 15EW; AE F032640 00 EW01 B106): content of a.s. deltamethrin: 1.51 % w/w
Batch number:	TA 161/99 PM
Reference item:	Zolone Flo SC 500 (500 g a.s./L nominal, analysed content: 499 g a.s./L)
Test organism:	Honey bees (*Apis mellifera L.*)

The used hives were single box colonies (type DADANT) with 10 frames, 10.000 bees and one queen at test start. Queens were obtained by grafting (1 month) and colonies (consisting of Caucasian bees) were homogeneous as possible. Oldest worker honeybees were a maximum of 3 months old at test initiation.

Furthermore the following criteria for each colony were guaranteed:

- 4 frames containing brood combs
- 2 frames for reserves
- 4 frames were kept empty for free space

Source: [redacted] (Supplier)

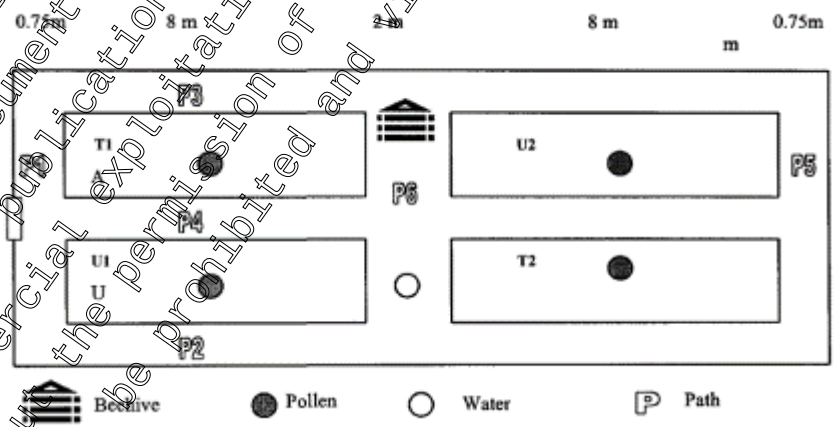
Crop: Winter wheat *(Triticum aestivum)* cultivar Thesee, sown October 03rd, 2000, Growth stage: BBCH 64-79

Test location: [redacted] France

Test unit: [redacted] France. The apiary part was conducted in [redacted]

The study was composed of 4 tunnels. Each tunnel was made of metal arches covered by a plastic netting. The ground area covered was approximately 136.5 m² (19.5 m x 7 m) and the height was approximately 3 m at the highest point of the arch. The tunnels were placed over the plots and with their long side perpendicular to the seed row after they had been sown with the crop. All tunnels were positioned with the same orientation regarding compass bearing. The diagram below shows the area within the tunnels, which was divided into four areas (T1, T2 and U1, U2) of 16 m² (8x2m) each. Areas T1 and T2 were areas that have received treatments while U1 and U2 were refuge zones not receiving any treatment.

The diagrammatic representation of a tunnel is shown in the following figure:



T1 and T2: treated areas
U1 and U2: refuge zones not receiving any treatment

	Areas were separated from each other by a path for observation. In each tunnel one hive was placed and assignment to tunnels was randomised.
Application rates:	Control (C): Mineral water ("Cristal Roc") Treatment rate 1: 0.417 L/ha (6.255 g a.s./ha) during foraging activity Treatment rate 2: 0.417 L/ha (6.255 g a.s./ha) during foraging activity Reference rate: 1.2 L/ha (600 g a.s./ha) Every treatment comprised of one replicate (i.e. 1 tunnel per treatment). The spray volume was 300 L/ha in all treatment groups. The sprayer was calibrated on the days of application. The deviation reached a maximum of 8.33%.
Data sampling:	Data for mortality, foraging activity, duration of flower visits, behaviour of the bees and data of the colony were assessed.
Data analysis:	Linear regression analysis using STAT-ITCF was done to compare the mortality of the bees during the acclimatisation phase and the mortality of the bees during the exposure period.
Deviations from the study plan:	One deviation was recorded in the study report. This deviation had no impact on the study because only the batch number was changed. The product was still Decis 15EW.

Climatic conditions during the experiment:

The environmental parameters recorded were within the normal range for the region. No dramatic weather conditions such as storms or violent winds occurred during the study period. The environmental conditions are shown in the table below.

Table 1: Field conditions

Date	Temperature Mini °C	Temperature Maxi °C	Relative air humidity % Mini	Relative air humidity % Maxi	Rainfall mm
01.06.01	9.1	21.7	36	85	-
02.06.01	9.4	20.6	46	98	0.2
03.06.01	4.5	19.2	33	95	-
04.06.01	6.1	22.5	31	97	-
05.06.01	9.8	25.0	27	88	-
06.06.01	10.5	21.6	51	92	-
07.06.01	12.0	21.6	45	93	2.8
08.06.01	10.0	16.9	67	97	0.2
09.06.01	8.5	20.4	46	89	-
10.06.01	6.0	21.1	28	82	-
11.06.01	6.9	22.2	31	96	-
12.06.01	9.0	25.6	31	97	-
13.06.01	10.2	26.2	26	86	-
14.06.01	12.6	24.2	39	99	10.4
15.06.01	12.5	20.8	53	99	0.6

Pesticide history of the field site:

Previous pesticide history of the test site is listed in the table below.

Table 2: Pesticide history of the field site

Year	Crop	Pesticides			
		Herbicides Name(a.i), rate	Fungicides Name(a.i), rate	Insecticides Name(a.i), rate	Other Name(a.i), rate
2000	Winter wheat (Thésée)	**Célio+Agral** (clodinafop+cloquintocet) 0.6+1l/ha	**Unix** (cyprodinil) 1kg/ha **Marathon** (cyproconazol+chlorothalonil) 2l/ha	**Gaucho blé** (imidacloprid+triticonazol+anthraquinone) 0.4l/100kg seeds **Karate** (lambda-cyalothrine) 0.125l/ha	**Terpal** (mepiquat+éthephon) 2l/ha
1999	Corn (Anjou 285)	**Gesaprime auto** (atrazine) 2l+0.8l/ha **Mikado** (sulcotrione) 0.7l/ha		**Gaucho** (imidacloprid) 0.071/50000 seeds **Karate** (lambda-cyalothrine) 0.125l/ha	
1998	Winter wheat (Cézanne)	**Starane 200+Allié** (fluroxypyr+metsulfuron-méthyle) 1L+0.015kgl/ha **Chardex** (clopyralid+oxyna-mêco-prop-p) 2L/ha	**Alto 100SL** (cyproconazol) 0.8l/ha **Caramba** (metconazole) 0.8l/ha	**Karate xpt** (lambda-cyalothrine) 0.125l/ha	**Cycocel C5** (chlormequat chlorure/chlorure de choline) 2L/ha

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EW 15 on the honeybee, *Apis mellifera* under forced exposure conditions.

This study included four exposure groups (tunnels) each: one tap-water treated control group, two test-item groups and one reference item group. In all exposure groups, the crop was sprayed 4 days after set-up of the hives in the tunnels (Acclimatisation phase) at BBCH 67 - 68 (full-flowering), during honeybees actively foraging on the crop under confined conditions. The honeybees remained 13 days in the tunnels.

Depending on the weather conditions the observations were conducted for a 7 day period following a 4-day adaptation period of the hives to the confinement. At the end of this 7 day period the exposure phase of the study was stopped and beehives returned to the apiary.

The assessments of the number of any dead bees were performed in each tunnel, each morning during both the adaptation period of the hives and thereafter until the end of the test. For each tunnel, these daily assessments was performed commencing June 07 in the morning and subsequent daily assessment was conducted at approximately the same time for each tunnel each day. During each assessment all dead bees were collected in the 6 paths and in the dead bee trap (the bees collected from each of the path areas 1 to 5 were pooled).

The assessments of the foraging activity were performed only on those days when the weather is such that honeybees are likely to be foraging. Counting was done over the whole of the zone by assessing the number of bees passing an area of 60cm wide, whilst walking at a normal speed down the side of the zone. The foraging activity of the bees was expressed as the number of bees observed separately within each zone on each assessment. After the adaptation period of the beehive, the foraging activity was evaluated twice a day at regular intervals (starting around 10 a.m. in the morning and 3 p.m. in the

afternoon). In addition, the day of treatment foraging activity is assessed around 15 minutes before treatment and around 15 minutes and 1 hour after each treatment.

The assessments of the number of bees leaving and entering the hive were performed within each tunnel and were recorded over a five minutes period. In order to avoid any mistake, counting of bees entering the hive was done for a 150 seconds period, then counting for bees leaving the hive for a 150 seconds period with another second sequence of 5 minutes.

The duration of flower visits by the bees was performed by recording the time (in seconds) that 15 different bees forage over 15 different attractive plants (This was done for 15 bees with a maximum time of 90 seconds in order not to delay the following assessments). The plants chosen for this assessment were chosen without conscious bias from those available within each tunnel.

Behaviour of bees was observed during assessment of bee mortality, foraging activity and control of the colony. Bees were observed for abnormalities like aggressiveness, intensive flying without landing on the crop, moribund or severely affected bees, bee accumulation at the beehive entrance, trembling bees no longer producing pollen balls, etc..

The following endpoints were assessed:

- Cumulative number of dead bees before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Number of foraging bees per zone (T1, T2 and U1, U2) and number of bee/ m^2 in each tunnel before as well as after the applications in the control, the test item groups and the reference item group, respectively.
- Number of bees leaving and entering the beehives and duration of flower visits in the control, the test item groups and the reference item group, respectively
- Behaviour of the bees during assessments assessment in the control, the test item groups and the reference item group, respectively
- Number of bees leaving and entering the hives in the control, the test item groups and the reference item group, respectively
- Duration of flower visits in the control, the test item groups and the reference item group, respectively
- Control of the colony with the following criteria examined: weight of the empty frame introduced into the centre of the hive, for both sides of each frame the percentage frame surface area containing honey, for both sides of each frame the percentage frame surface area containing pollen, for both sides of each frame the percentage frame surface area containing eggs, for both sides of each frame the percentage surface area of brood (young and old larvae) in each frame and % of capped and uncapped alveolus as well as the health of the queen.

Dates of Work: 5th June to 22nd June 2001

Findings:

Honey Bee Mortality

A summary of the daily mortality (cumulated dead bees) is shown in following table.

Table 3: Cumulated dead bees during the study period (only males and worker-bees considered)

Date	Tunnel No. 1 Deltamethrin EW 15 @ 6.3 g a.s./ha			Tunnel No. 2 Deltamethrin EW 15 @ 6.3 g a.s./ha			Tunnel No. 3 Zolone Flo @ 600 g a.s./ha			Tunnel No. 4 Water		
	Males	Workers	Total	Males	Workers	Total	Males	Workers	Total	Males	Workers	Total
07.06.01 2DBT	10	1105	1115	11	933	944	3	607	610	6	1150	1156
08.06.01 1DBT	18	1220	1238	15	1164	1179	13	776	789	11	1314	1325
09.06.01 0DBT	18	1243	1261	19	1207	1226	16	804	820	14	1345	1359
10.06.01 1DAT	19	1375	1394	26	1435	1461	23	874	987	25	1378	1400
11.06.01 2DAT	20	1590	1610	58	1789	1847	25	1085	1110	28	1453	1481
12.06.01 3DAT	20	1760	1780	67	1988	2055	26	1276	1302	38	1517	1555
13.06.01 4DAT	20	1835	1855	75	2153	2228	30	1431	1461	57	1561	1618
14.06.01 5DAT	20	1973	1993	95	2341	2438	33	1669	1702	61	1644	1705
15.06.01 6DAT	20	2037	2057	102	2426	2528	35	1771	1806	61	1683	1744

DBT = days before treatment; DAT = days after treatment

The effect of Deltamethrin EW 15 on bee mortality was similar to the effect on the bee mortality of the non-toxic standard Zolone Flo, regardless whether tunnel of the two treatment groups (No. l or No. 2).

Honey Bee Foraging activity

Deltamethrin EW 15 had no or very limited effect on the foraging activity in the days following the treatment on both treated and refuges areas.

Table 4: Number of bees foraging in the treated zones (T1, T2) in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) and Zolone Flo.

Assessment date (Day/month/hour)	Number of bees/m² (means)			
	Tunnel N°4 Water	Tunnel N°1 AE F032640 00 EW01 B106	Tunnel N°2 AE F032640 00 EW01 B106	Tunnel N°3 Zolone Flo
08/06 - 14h45-15h05	4,00	3,08	2,47	3,44
08/06 - 16h57-17h21	3,81	3,28	2,66	2,53
09/06 - 10h01-10h24	3,84	3,63	2,69	2,44
09/06 - 10h52-11h37	3,44	5,05	2,88	3,26
09/06 - 11h45-12h25	1,78	1,28	0,47	0,52
09/06 - 12h56-13h23	0,41	0,07	0,09	0,14
09/06 - 15h57-16h17	1,06	0,33	0,44	1,38
10/06 - 11h07-11h27	2,97	0,64	0,41	0,17
10/06 - 15h02-16h23	0,56	0,17	0,47	0,10
11/06 - 10h07-10h29	4,56	1,31	0,94	0,69
11/06 - 15h30-15h52	1,63	1,38	1,84	0,25
12/06 - 09h57-10h19	4,78	3,57	1,91	1,40
12/06 - 16h37-16h58	0,50	2,05	1,72	0,72
13/06 - 10h47-11h09	3,41	4,58	3,38	0,23
13/06 - 16h17-16h42	1,00	1,22	1,16	0,58
14/06 - 11h02-11h27	7,81	7,87	3,72	2,52
14/06 - 15h49-16h12	0,25	1,87	3,31	0,31
15/06 - 11h17-11h39	4,09	7,72	2,78	2,00
15/06 - 15h33-15h54	1,78	6,01	5,31	0,69
08/06 - 14h45-15h05	4,00	3,08	2,47	3,44

Treatments

Table 5: Number of bees foraging in the refuge zones (U1, U2) in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) and Zolone Flo.

Assessment date (Day/month/hour)	Number of bees/m² (means)			
	Tunnel N°4 Water	Tunnel N°1 AE F032640 00 EW01 B106	Tunnel N°2 AE F032640 00 EW01 B106	Tunnel N°3 Zolone Flo
08/06 - 14h45-15h05	3,66	3,17	2,71	3,48
08/06 - 16h57-17h21	3,81	4,35	2,42	2,68
09/06 - 10h01-10h24	4,44	3,24	3,04	2,86
09/06 - 10h52-11h37	4,19	4,19	2,38	3,89
09/06 - 11h45-12h25	1,84	2,28	1,91	0,93
09/06 - 12h56-13h23	0,34	0,66	0,20	0,60
09/06 - 15h57-16h17	1,72	1,58	1,78	2,58
10/06 - 11h07-11h27	2,22	1,99	1,35	0,51
10/06 - 15h02-16h23	1,16	0,59	2,19	0,19
11/06 - 10h07-10h29	5,84	4,65	4,53	1,36
11/06 - 15h30-15h52	3,00	3,09	2,11	0,46
12/06 - 09h57-10h19	5,84	4,87	5,29	2,71
12/06 - 16h37-16h58	0,91	3,43	3,35	1,05
13/06 - 10h47-11h09	3,78	7,11	5,90	0,90
13/06 - 16h17-16h42	1,19	4,17	3,20	0,90
14/06 - 11h02-11h27	8,50	10,95	11,14	5,16
14/06 - 15h49-16h12	0,31	1,04	2,94	0,64
15/06 - 11h17-11h39	4,84	6,72	6,90	4,15
15/06 - 15h33-15h54	2,00	4,37	5,06	0,92
08/06 - 14h45-15h05	3,66	3,17	2,71	3,48

Treatments

Bees leaving and entering the beehives

Deltamethrin EW 15 had not shown any effect different from the non-toxic standard Zolone Flo on the number of bees leaving and entering the beehive just after the treatment.

Table 6: Bees leaving and entering the beehives

Assessment date (Day/month/hour)	Number of bees/m² (means)							
	Tunnel N°4 Water		Tunnel N°1 AE F032640 00 EW01 B106		Tunnel N°2 AE F032640 00 EW01 B106		Tunnel N°3 Zolone Flo	
	Leaving	Entering	Leaving	Entering	Leaving	Entering	Leaving	Entering
08/06 - 14h45-15h44	141,00	175,00	93,00	122,00	89,00	105,00	101,00	132,00
09/06 - 15h57-16h17	294,00	311,00	198,00	206,00	264,00	264,00	130,00	162,00
10/06 - 11h07-11h27	104,00	118,00	118,00	108,00	41,00	63,00	80,00	88,00
10/06 - 15h02-15h23	185,00	174,00	148,00	196,00	142,00	161,00	131,00	150,00
11/06 - 10h07-10h29	160,00	140,00	122,00	129,00	82,00	104,00	141,00	154,00
11/06 - 15h30-15h52	125,00	176,00	288,00	351,00	133,00	144,00	195,00	185,00
12/06 - 09h57-10h19	117,00	149,00	232,00	243,00	96,00	129,00	210,00	247,00
12/06 - 16h37-16h58	243,00	221,00	209,00	237,00	164,00	187,00	269,00	195,00
13/06 - 10h47-11h09	135,00	149,00	207,00	239,00	153,00	157,00	182,00	169,00
14/06 - 11h02-11h27	250,00	329,00	333,00	337,00	215,00	274,00	341,00	299,00

Treatments [redacted]

Duration of flower visits

The results did not show any effect of Deltamethrin EW 15 on the duration of flower visits.

Table 7 Duration of flower visits (based on 15 bees)

Assessment date (Day/month/hour)	Total in seconds (based on 15 bees)			
	Tunnel N° Water	Tunnel N°1 AE F032640 00 EW01 B106	Tunnel N°2 AE F032640 00 EW01 B106	Tunnel N°3 Zolone Flo
08/06 - 14h45-15h06	224,71	255,53	279,85	305,58
09/06 - 15h57-16h17	110,30	161,89	157,70	221,08
10/06 - 15h02-15h23	244,23	276,58	147,65	400,42
11/06 - 10h07-10h29	223,76	144,51	116,41	155,31
11/06 - 15h30-15h52	207,60	148,19	184,61	335,78
12/06 - 09h57-10h19	268,64	182,47	140,63	233,89
12/06 - 16h37-16h58	263,60	334,20	114,76	254,68
13/06 - 10h47-11h12	180,85	249,10	249,17	136,72
14/06 - 11h02-11h27	169,57	169,17	213,41	111,99

Treatments [redacted]

Behaviour of the bees

Deltamethrin EW 15 had no effect on the bee behaviour in the days following the treatment and in the days after beehives returned to the apiary.

Control of the colony

Reserves and brood were reduced during the study in all the tunnels, which is typical of such a study. Deltamethrin EW 15 had no negative effect on the control of the colony and this at each date of assessment.

Assessments of the control of the colony are listed in the following tables.

Table 8: Control of the colony exposed to water treated wheat (Tunnel No. 4)

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6
Weight in g		-	-	-	-	-	-	-	-	-	-	-	600	600	
% frame surface area containing honey	Side a	30	10	25	10	0	20	20	15	10	15	50	20	0	0
	Side b	30	10	20	10	0	30	10	50	10	15	50	15	0	0
% frame surface area containing pollen	Side a	0	0	15	0	0	0	0	0	0	0	30	15	0	0
	Side b	0	0	5	0	0	0	0	0	0	0	20	10	0	0
% frame surface area containing eggs	Side a	NR	0	NR	5	0	0	NR	10	10	0	NR	10	NR	NR
	Side b	NR	0	NR	0	0	0	NR	10	60	0	NR	15	NR	NR
% surface area of brood	Side a	0	0	0	0	20	15	90	70	30	50	0	20	0	0
	Side b	0	0	0	0	20	0	80	50	60	30	0	30	0	0
% capped alveolus	Side a	NR	NR	NR	NR	0	0	100	50	90	30	NR	50	NR	NR
	Side b	NR	NR	NR	NR	0	NR	100	60	30	50	NR	50	NR	NR
% uncapped alveolus	Side a	NR	NR	NR	NR	100	100	0	50	10	70	NR	50	NR	NR
	Side b	NR	NR	NR	NR	100	NR	0	40	60	50	NR	50	NR	NR
		T	T	T	T	T	T	T	T	T	T	T	T	T	T

NR = Not relevant, T = Treatment

Table 9: Control of the colony exposed to Deltamethrin EW 15 treated wheat (Tunnel No. 1)

Oservations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	800	800
% frame surface area containing honey	Side a	20	10	5	0	10	10	0	5	10	10	10	0	5	0
	Side b	10	10	5	5	10	10	0	5	10	15	5	0	0	0
% frame surface area containing pollen	Side a	5	0	20	10	0	0	0	0	0	0	0	0	0	0
	Side b	5	0	20	0	1	0	0	0	0	0	1	0	0	0
% frame surface area containing eggs	Side a	NR	NR	0	0	0	NR	NR	NR	0	5	NR	NR	NR	NR
	Side b	NR	NR	0	NR	0	0	NR	NR	0	0	10	5	NR	NR
% surface area of brood	Side a	0	0	50	80	60	60	80	40	50	30	30	40	0	0
	Side b	0	0	50	60	50	60	80	50	50	40	30	40	0	0
% capped alveolus	Side a	NR	NR	5	80	85	100	100	100	80	90	100	100	NR	NR
	Side b	NR	NR	90	100	80	95	100	100	90	90	90	90	NR	NR
% uncapped alveolus	Side a	NR	NR	95	20	15	0	0	0	20	10	0	0	NR	NR
	Side b	NR	NR	0	0	20	5	0	0	10	10	10	10	NR	NR
		T		T		T		T		T		T		T	

NR = Not relevant, T = Treatment

Table 10: Control of the colony exposed to Deltamethrin EW 15 treated wheat (Tunnel No. 2)

Observations	Dates	Frame N°1		Frame N°2		Frame N°3		Frame N°4		Frame N°5		Frame N°6		Empty Frame	
		05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6	05.6	15.6
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	550	500
% frame surface area containing honey	Side a	30	15	20	15	15	10	10	5	10	10	10	10	10	0
	Side b	20	10	15	10	15	10	10	5	10	10	15	10	10	0
% frame surface area containing pollen	Side a	15	0	15	0	0	0	0	0	0	0	3	0	0	0
	Side b	0	0	1	0	0	0	0	0	0	0	10	0	0	0
% frame surface area containing eggs	Side a	NR	NR	0	0	0	NR	10	0	0	0	0	NR	NR	NR
	Side b	NR	NR	NR	NR	0	NR	10	5	0	NR	0	NR	NR	NR
% surface area of brood	Side a	0	0	20	5	70	50	80	60	70	60	50	30	0	0
	Side b	0	0	0	0	70	50	90	80	70	60	40	30	0	0
% capped alveolus	Side a	NR	NR	0	100	95	100	90	50	90	80	80	100	NR	NR
	Side b	NR	NR	NR	NR	95	100	90	50	90	100	90	100	NR	NR
% uncapped alveolus	Side a	NR	NR	100	0	5	0	10	50	10	20	20	0	NR	NR
	Side b	NR	NR	NR	NR	5	0	10	50	10	0	10	0	NR	NR
		T		T		T		T		T		T		T	

NR = Not relevant, T = Treatment

Conclusion:

The effect of Deltamethrin EW 15 on bee mortality was similar to the effect on the bee mortality of the non-toxic standard Zolone Flo. Deltamethrin EW 15 had no or very limited effect on the foraging activity in the days following the treatment on both treated and refuges areas. Deltamethrin EW 15 had not shown any effect different from the nontoxic standard Zolone flo on the number of bees leaving and entering the beehive just after the treatment. The results did not show any effect of Deltamethrin EW 15 on the duration of flower visits. Deltamethrin EW 15 had no effect on the bee behaviour in the days following the treatment and in the month after beehives returned to the apiary. Reserves and brood were

reduced during the study in all the tunnels. Deltamethrin EW 15 had no negative effect on the control of the colony and this at each date of assessment.

*****

Report:	**KCP 10.3.1.5/13, [redacted]; 2001**
Title:	Tunnel test - acute, short and medium term effects of AE F032640 00 EW01 B106, applied on cereals, on honey bees (*Apis mellifera* L.)
Document No:	M-205201-01-1 (Rep. No.: S00AGB3264VO56)
Guidelines:	EPPO 170, (1992), CEB 129
GLP:	yes

~~**Material and Methods:**~~

~~Bees were confined within tunnels on winter wheat fields (cereals sprayed with sugar solution in order to provide food resources to the bees). After an acclimatization phase of four days, application was performed during bee flight. The control was treated with water, the test item was applied at a rate of 0.42 L/ha, as a non-toxic standard, Zolone Flo was used at a rate of 1.2 L/ha. There was one replicate per treatment group. Endpoints assessed were mortality, foraging and flight activity, storage of honey and pollen, behavior, and brood development.~~

~~**Findings:**~~

~~Mortality was not affected by the test substance treatment. Foraging activity was likewise not or only very slightly affected by the test substance treatment on the treated as well as on the refuge areas in the tunnel. Furthermore, no effects on the behavior were detected. Brood development was not affected by the test substance treatment as well.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EW 15 (AE F032640 00 EW01 B106) content of a.s.: deltamethrin: 16.14 g/L, 1.51 % w/w.
Batch number:	06
Reference item:	Zolone Flo SC 500 (500 g a.s./L nominal, analysed content: 510 g a.s./L)
Test organism:	Honey bees (*Apis mellifera*) The used hives were single box colonies (type DADANT 10 frames) with 10 frames, one queen and about 10000 bees per hive at test start. Queens were obtained by grafting (1 month) and colonies (consisting of Caucasian bees) were homogeneous as possible. Oldest worker honeybees were a maximum of 3 months old at test initiation. Additionally, an empty new frame of knowing weight was introduced in each hive prior their introduction into the tunnels. The corresponding queens hatched in 2000 and originated from one breeding line in order to guarantee uniform bee material in all treatment groups. Furthermore the following criteria for each colony were guaranteed:

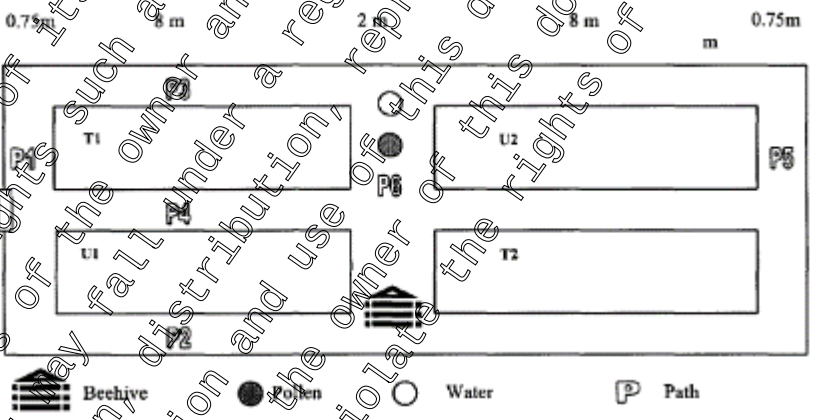
• 4 frames containing eggs, larvae and capped cells
• 2 frames containing honey and pollen
• 4 frames were kept empty for free space

Source: [redacted] (Supplier)

Crop: Winter wheat, cultivar Soissons sown October 21, 1999. Growth stage: BBCH 75-77

Test location: [redacted] France, [redacted] France. The apiary part was conducted [redacted]

Test unit: Each tunnel covered an area of 136.5 m² (19.5 m × 7 m) with a height of approximately 3.5 m. The tunnel tent frames were covered with light plastic netting. Furthermore, each tunnel was divided into four areas (T1, T2 and U1, U2) of 16 m² (8 m × 2 m) each, containing the crop. Areas T1 and T2 received treatment while U1 and U2 were refuge zones not receiving any treatment. The diagrammatic representation of a tunnel is shown in the following figure:



T1 and T2: treated areas
U1 and U2: refuge zones not receiving any treatment

Areas were separated from each other by a path for observation. In each tunnel one hive was placed and assignment to tunnels was randomised.

Application rates: Control (C): mineral water (Clarine)

Treatment rate (T): 0.42 L/ha (6.3 g a.s./ha) during foraging activity

Reference rate (R): 1.2 L/ha (600 g a.s./ha)

Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).

	The spray volume was 300 L/ha in all treatment groups. The sprayer was calibrated before use. The deviation reached a maximum of 4.17%.
Data sampling:	Data for mortality, foraging activity, behaviour of the bees and data of the control colony were assessed.
Data analysis:	Not stated in the report.
Deviations from the study plan:	No deviation to the study protocol.

Climatic conditions during the experiment:

The environmental parameters recorded were within the normal range for the region. No dramatic weather conditions such as storms or violent winds occurred during study period. The environmental conditions are shown in table below.

Table 1: Field conditions

Date	Temperature Mini °C	Temperature Maxi °C	Relative air humidity % Mini	Relative air humidity % Maxi	Rainfall mm
10.05.00	14.6	23.7	54	97	3.8
11.05.00	14.3	22.2	62	98	0.8
12.05.00	12.8	23.5	48	98	-
13.05.00	11.1	23	51	98	-
14.05.00	12.5	26.7	45	99	-
15.05.00	14.7	27.8	42	95	-
16.05.00	11.6	25	56	95	-
17.05.00	8.9	17.5	45	92	-
18.05.00	7.7	17.4	48	94	2.2
19.05.00	3.4	15.5	53	95	-
20.05.00	5.1	18.2	37	99	-
21.05.00	4.8	17.2	48	96	-
22.05.00	7.8	18.8	49	98	-
23.05.00	8.5	21.5	49	99	-
24.05.00	11.7	21.8	44	91	0.2
25.05.00	7.1	18.5	43	96	-
26.05.00	7.2	20.9	49	94	-
27.05.00	7.9	18.3	35	91	2.6
28.05.00	4.2	17.9	38	96	-
29.05.00	7.2	17.7	40	98	1
30.05.00	11.6	16.4	83	98	14.6
31.05.00	10.8	19.2	63	98	-
01.06.00	13	22.4	63	97	-
02.06.00	14.5	29.2	54	98	-
03.06.00	17.2	25.4	69	98	0.4
04.06.00	12.9	21.5	63	97	12.4
05.06.00	9.7	14.2	72	96	3.6
06.06.00	8.4	18.5	56	98	-
07.06.00	9.4	21.1	41	98	-
08.06.00	11.2	26.9	37	90	-
09.06.00	12.6	24.9	49	97	12.8
10.06.00	9.6	18.9	59	96	-
11.06.00	8.1	20	47	97	-
12.06.00	10	24.5	53	98	-
13.06.00	15.3	27.7	46	98	-
14.06.00	14	25.7	53	97	-
15.06.00	14.8	27.6	61	98	-
16.06.00	12.7	25.4	50	99	-
17.06.00	11	26.3	37	89	-
18.06.00	13.2	31.1	32	87	-
19.06.00	17.3	32.1	37	96	-
20.06.00	15.8	30.7	55	95	-
21.06.00	13	24.8	52	96	-
22.06.00	12.7	23.2	38	93	-
23.06.00	13	21.2	46	90	-
24.06.00	11	18.3	44	94	-
25.06.00	9.9	20.9	34	90	-
26.06.00	9.4	22.6	33	90	-
27.06.00	11.3	23.5	31	83	0.4
28.06.00	12.1	25.3	31	82	-
29.06.00	12.6	26.2	33	86	-
30.06.00	14.4	28.5	41	92	-

Pesticide history of the field site:

Previous pesticide history of the test site is listed in the following table.

Table 2: Maintanance and pesticide history of the field site

Year	Crop	Pesticides			
		Herbicides Name (a.i.), rate	**Fungicides** Name (a.i.), rate	**Insecticides** Name (a.i.), rate	**Other**
2000	Winter wheat	**Celio** (March 7) (clodinafop-propargyl+ cloquincet-methyl) 0.6 L/ha **Agral 90** (March 7) 1 L/ha	**Unix** (April 18) (cyprodinil) 1 kg/ha **Amistar** (May 9) (azoxystrobine) 0.8 L/ha **Ogam** (May 26) (kresoxim-methyl+epoxiconazol) 0.8 L/ha	**Karate vert** (May 9) (lambda-cyhalothrin) 0.125 L/ha	
1999	Winter wheat	**Starane** (fluroxypyr) 0.5 L/ha **Chardax** (clopyralid+2,4-MCPA) 1.5 L/ha	**Ogam** (kresoxim-methyl+epoxiconazol) 0.6 L/ha **Caramba** (metconazole) 1 L/ha	**Gaucho blé** (imidacloprid+ bitertanol+anthraquinone) 0.4 L/100 kg seeds **Karate vert** (lambda-cyhalothrin) 0.125 L/ha	-
1998	Corn	**Gesaprime** (atrazine) 3 L/ha **Mikado** (sulcotrione) 0.8 L/ha		**Gaucho** (imidacloprid) 0.67kg/150000 seeds **Karate** (lambda-cyhalothrin) 0.125 L/ha	-
1997	Winter wheat	**Starane** (fluroxypyr) 0.5 L/ha **Chardax** (clopyralid+2,4-MCPA) 1.5 L/ha	**Alto** (cyptoconazol) 0.8 L/ha **Alto Marathon** (cyproconazole+chlorothalonil) 2 L/ha	**Gaucho blé** (imidacloprid+ bitertanol+ anthraquinone) 0.4 L/100 kg seeds **Karate vert** (lambda-cyhalothrin) 0.125 L/ha	-

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EW 15 on the honeybee *Apis mellifera* under forced exposure conditions.

This study included three exposure groups (tunnels) each: one tap-water treated control group (C), one test item group (T) and one reference item group (R). In all exposure groups, the crop was sprayed 5 days after set-up of the hives in the tunnels (Acclimatisation phase) at BBCH 75 - 77 (full-flowering), during honeybees actively foraging on the crop under confined conditions. The honeybees remained 13 days in the tunnels.

Depending on the weather conditions the observations were conducted for a 9 day period following a 4-day adaptation period of the hives to the confinement. At the end of this 9 day period, symptoms of toxicity (mortality, behaviour, etc.) were not observed in the Deltamethrin EW 15 treatment, the exposure phase of the study was stopped and beehives returned to the apiary.

The assessments of the number of any dead bees were performed in each tunnel, each morning during both the adaptation period of the hives and thereafter until the end of the test. For each tunnel, these daily assessments was performed commencing June 19 at approximately 36 hours after the introduction of the hive and subsequent daily assessment was conducted at approximately the same time for each tunnel each day. During each assessment all dead bees were collected in the 6 paths and in the dead bee trap (the bees collected from each of the path areas 1 to 5 were pooled).

The assessments of the foraging activity were performed only on those days when the weather is such that honeybees are likely to be foraging. Counting was done over the whole of the zone by assessing the number of bees passing an area of 60cm wide, whilst walking at a normal speed down the side of the zone. The foraging activity of the bees was expressed as the number of bees observed separately within each zone on each assessment. After the adaptation period of the beehive, the foraging activity was evaluated twice a day at regular intervals (starting around 10 a.m. in the morning and 3 p.m. in the afternoon). In addition, the day of treatment foraging activity is assessed around 15 minutes before treatment and around 15 minutes and 1 hour after each treatment.

Behaviour of bees was observed during assessment of bee mortality, foraging activity and control of the colony. Bees were observed for abnormalities like aggressiveness, intensive flying without landing on the crop, moribund or severely affected bees, bee accumulation at the beehive entrance, trembling, bees no longer producing pollen balls, etc.

Assessments on the control of the colony were made on the day of their installation within the tunnels, June 17, on the middle of the exposure phase, June 26, just after returning bee hives to the beekeeper, June 30, and around one month after the return to the apiary, August 8.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Cumulative number of dead bees before as well as after the applications in the control, the test item group and the reference item group, respectively
- Number of foraging bees per zone (T1, T2 and U1, U2) and number of bee/m^2 in each tunnel before as well as after the applications in the control, the test item group and the reference item group, respectively.
- Behaviour of the bees during assessments of bee mortality, foraging activity and the control of the colony. In addition the date time and duration of such abnormal behaviours was recorded.
- Control of the colony with the following criteria examined: weight of the empty frame introduced into the centre of the hive, for both sides of each frame the percentage frame surface area containing honey, for both sides of each frame the percentage frame surface area containing pollen, for both sides of each frame the percentage frame surface area containing eggs, for both sides of each frame the percentage surface area of brood (young and old larvae) in each frame and % of capped and uncapped alveolus as well as the health of the queen.

Dates of Work: 17th June to 08th August, 2000

Findings:

Honey bee mortality

A summary of the daily mortality (cumulated dead bees) is shown in following table.

Table 3: Cumulated dead bees during the study period (only males and worker-bees considered)

Date	Tunnel No. 5 Deltamethrin EW 15 @ 6.3 g a.s./ha			Tunnel No. 2 Water			Tunnel No. 4 Zolone Flo @ 600 g a.s./ha		
	Males	Workers	Total	Males	Workers	Total	Males	Workers	Total
19.06.00 3DBT	29	1109	**1138**	12	244	**256**	14	890	**904**
20.06.00 2DBT	49	1255	**1304**	27	423	**450**	23	1162	**1185**
21.06.00 1DBT	86	1359	**1445**	30	475	**505**	56	1294	**1350**
22.06.00 0DBT	91	1426	**1517**	33	494	**527**	66	1400	**1466**
22.06.00 0DAT	96	1474	**1570**	33	513	**546**	73	1466	**1539**
23.06.00 1DAT	100	1481	**1581**	34	524	**558**	79	1478	**1557**
24.06.00 2DAT	110	1533	**1643**	35	551	**586**	86	1788	**1874**
26.06.00 4DAT	110	1585	**1695**	41	692	**733**	119	2099	**2218**
27.06.00 5DAT	111	1675	**1786**	42	812	**854**	138	2425	**2563**
28.06.00 6DAT	114	1755	**1869**	43	938	**981**	140	2622	**2762**
29.06.00 7DAT	118	1834	**1952**	44	957	**1001**	141	2905	**3046**
30.06.00 8DAT	118	1876	**1994**	44	999	**1043**	141	3143	**3284**

DBT = days before treatment, DAT = days after treatment

The effect of Deltamethrin EW 15 on bee mortality was nil and even lower than the bee mortality of the non-toxic standard Zolone Flo for which a slight increase in mortality was observed two to three days after application. When beehives returned to the apiary no mortality was observed.

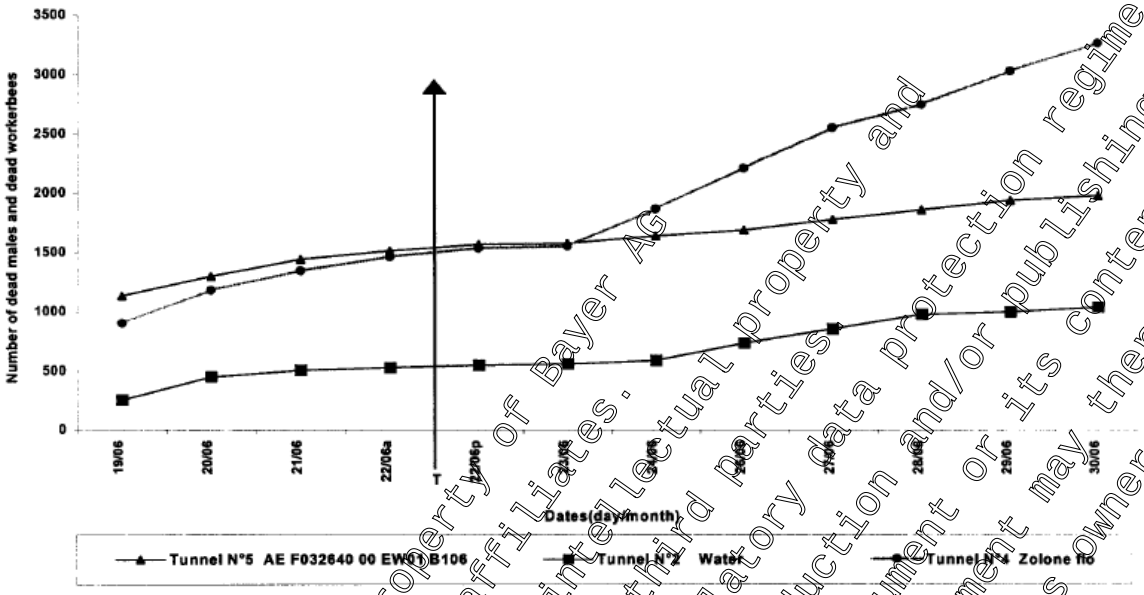


Figure 1: Cumulative mortality of bees (T= Treatment) in the different tunnels: water control, Deltamethrin EC 15 (AE F032640 00 EW01 B106) and Zolone Flo.

Honey bee flight intensity

Foraging activity in cereals was basically low in all tunnels. Deltamethrin EW 15 had no or limited effect on the foraging activity in the days following the treatment on both treated and refuges areas.

Comparisons between the different treatments and treated zones and refuge zones are presented in the following tables.

Table 4: Number of bees foraging in the treated zones (T1, T2) in the different tunnels: Deltamethrin EW 15 (AE F032640 00 EW01 B106), water control and Zolone flo

Assessment date (Day/month/hour)	Number of bees/m² (means)		
	Tunnel N°5 AE F032640 00 EW01 B106	Tunnel N°2 Water	Tunnel N°4 Zolone flo
21/06/10h11-10h35	1.16	1.41	1.50
21/06/15h05- 15h31	3.06	1.06	2.91
22/06/09h50-10h24	3.97	2.41	5.66
22/06/11h28-12h30	1.88	1.38	1.44
Treatments			
22/06/12h00-13h05	0.72	0.97	0.38
22/06/12h57-13h46	0.34	0.88	0.38
22/06/16h16-16h35	0.06	0.16	0.50
23/06/10h15-10h40	2.34	2.31	1.94
23/06/15h08-15h31	0.47	2.53	1.09
24/06/10h18-11h17	1.31	1.25	1.13
26/06/10h08-10h34	1.38	1.78	1.44
26/06/15h51-16h15	0.88	1.31	1.91
27/06/10h04-10h28	2.22	2.97	1.94
27/06/15h09-15h36	1.97	3.56	3.13
28/06/10h11-10h49	8.59	6.84	8.09
28/06/16h11-16h37	2.03	2.88	1.28
29/06/10h59-11h26	2.00	2.00	0.97
29/06/15h50-16h13	0.84	1.34	1.31
30/06/10h28-10h56	6.31	7.47	3.13
30/06/15h15-15h37	0.81	0.59	0.75

Table 5: Number of bees foraging in the refuge zones (U1, U2) in the different tunnels: Deltamethrin EW 15 (AE F032640 00 EW01 B106), water control and Zolone flo

Assessment date (Day/month/hour)	Number of bees/m² (means)		
	Tunnel N°5 AE F032640 00 EW01 B106	Tunnel N°2 Water	Tunnel N°4 Zolone flo
21/06/10h11-10h35	1.31	2.03	1.66
21/06/15h05- 15h31	2.50	0.94	3.47
22/06/09h50-10h24	4.41	2.13	5.22
22/06/11h28-12h30	1.94	1.69	1.16
Treatments			
22/06/12h00-13h05	1.75	1.06	1.34
22/06/12h57-13h46	0.66	0.56	0.50
22/06/16h16-16h35	0.94	0.38	0.75
23/06/10h15-10h40	1.09	1.94	3.13
23/06/15h08-15h31	1.94	2.56	1.47
24/06/10h18-11h17	1.16	1.25	1.16
26/06/10h08-10h34	1.41	1.56	1.28
26/06/15h51-16h15	1.22	1.25	1.72
27/06/10h04-10h28	2.59	3.00	2.19
27/06/15h09-15h36	4.19	2.97	4.13
28/06/10h11-10h49	7.34	6.59	6.44
28/06/16h11-16h37	1.66	1.81	1.63
29/06/10h59-11h26	1.91	1.47	0.78
29/06/15h50-16h13	0.84	1.59	0.69
30/06/10h28-10h56	7.56	6.03	3.28
30/06/15h15-15h37	0.59	0.72	1.44

Behaviour of the bees

Deltamethrin EW 15 had no effect on the bee behaviour in the days following the treatment and in the month after beehives returned to the apiary.

Control of the colony

Reserves and brood were reduced during the study in all the tunnels, which is typical of such a study. Deltamethrin EW 15 had no negative effect on the control of the colony and this at each date of assessment.

Assessments of the control of the colony are listed in the following tables.

Table 6: Control of the colony exposed to Deltamethrin EW 15 treated wheat

Observations		Frame N°1 N° VO56-1-C1				Frame N°2 N° VO56-1-C2				Frame N°3 N° VO56-1-C3				Frame N°4 N° VO56-1-C4				Frame N°5 N° VO56-1-C5				Frame N°6 N° VO56-1-C6				Empty frame			
Date		17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1300	-	1500	2500
% frame surface area containing honey	Side a	0	10	0	75	5-10	10	25	40	15	15	5-10	30	15	15	15-10	30	10	15	5-10	30	50	50	60-70	80	0	0	0	50
	Side b	15	15	20	60	5	10	15-20	40	10	10	5-10	30	10	10	15-10	30	20	20	5-10	20-30	50	0	0	80	0	0	0	60
% frame surface area containing pollen	Side a	0	0	0	10	20	10	0	10	10	5	0	10	10	10	0	10	0	0	0	5	0	0	0	0	0	0	0	10
	Side b	20	0	0	10	10	5	0	2-5	5	5	0	10	10	10	0	10	0	0	0	15	0	0	0	0	0	0	0	10
% frame surface area containing eggs	Side a	NR	NR	NR	NR	NR	NR	NR	2-5	NR	0	NR	0	NR	0	0	30	NR	0	0	NR	NR	NR	NR	NR	NR	NR	NR	5
	Side b	NR	NR	NR	0	1-2	NR	NR	0	NR	0	NR	5	NR	0	0	5	NR	0	0	80	NR	NR	NR	NR	NR	NR	NR	5
% surface area of brood	Side a	0	0	0	5	50	50	20	40	70	50	20	60	80	50	80	30	70	60	80	30	0	0	0	0	0	0	0	10-20
	Side b	0	0	0	5	15	15	20	40	80	50	10	60	80	50	80	40	80	80	40	60	0	0	0	0	0	0	0	10-20
% capped alveolus	Side a	NR	NR	NR	100	0	100	100	80	60	60	100	95	75	10	30	0	20	50	10	100	NR	NR	NR	NR	NR	NR	NR	0
	Side b	NR	NR	NR	0	0	100	100	90	50	50	100	70	90	10	30	0	90	50	0	0	NR	NR	NR	NR	NR	NR	NR	0
% uncapped alveolus	Side a	NR	NR	NR	0	100	0	0	20	40	40	0	0	25	80	70	100	20	50	80	0	NR	NR	NR	NR	NR	NR	NR	100
	Side b	NR	NR	NR	100	100	0	0	10	50	50	0	30	10	80	70	90	10	50	100	100	NR	NR	NR	NR	NR	NR	NR	100
		T				T				T				T				T				T				T			

NR: Not relevant; T: Treatment

Table 7: Control of the colony exposed to the water treated wheat

Observations		Frame N°1 N° VO54-1-C1				Frame N°2 N° VO54-1-C2				Frame N°3 N° VO54-1-C3				Frame N°4 N° VO54-1-C4				Frame N°5 N° VO54-1-C5				Frame N°6 N° VO54-1-C6				Empty frame			
Date		17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	750	-	750	2000
% frame surface area containing honey	Side a	10	15	0	60	10	10	30	20	15	15	0	30	5-10	15	5-15	30	15	15	30	30	15	15	5-10	30	0	0	0	70
	Side b	0	10	50	30	10	10	50	30	10	15	10	30	10	10	10-15	30	10	20	10	30	15	15	5-10	30	0	0	0	80
% frame surface area containing pollen	Side a	0	0	0	10	10	10	0	5	30	5	0	10	0	0	0	5	0	0	0	15	0	10	30	15	0	0	0	20
	Side b	10	0	0	0	20	0	0	0	20	5	0	5	0	5	0	5	0	0	0	0	0	15	80	0	0	0	0	0
% frame surface area containing eggs	Side a	NR	NR	NR	NR	5	2-5	NR	NR	5	0	10	80	5-10	0	10	0	10	0	NR	0	NR	NR	NR	0	NR	NR	NR	NR
	Side b	NR	NR	NR	0	NR	0	NR	NR	NR	0	10	60	NR	0	0	0	NR	0	NR	0	NR	NR	NR	10	NR	NR	NR	NR
% surface area of brood	Side a	0	0	0	0	30	30	10	70-60	50	50	80	50	50	50	80	70	15	15	30	60	5-10	5	0	60	0	0	0	0
	Side b	0	0	0	60	30	20	10	60	80	60	80	50	60	60	80	50-60	0	10	20-30	50-60	0	0	0	70	0	0	0	0
% capped alveolus	Side a	NR	NR	NR	NR	50	50	100	100	80	80	0	0	80	80	80	70	0	80	100	90	60	100	NR	70	NR	NR	NR	NR
	Side b	NR	NR	NR	20	60	60	100	100	60	60	0	0	50	50	80	10-15	NR	80	100	90	50	NR	NR	10	NR	NR	NR	NR
% uncapped alveolus	Side a	NR	NR	NR	NR	45	45	0	0	10	20	100	100	10	10	20	30	100	10	0	5	40	0	NR	30	NR	NR	NR	NR
	Side b	NR	NR	NR	80	40	40	0	0	40	40	100	100	50	50	20	80	NR	20	0	10	50	NR	NR	90	NR	NR	NR	NR
		T				T				T				T				T				T				T			

NR: Not relevant; T: Treatment

Conclusion:

The effect of Deltamethrin EW 15 on bee mortality was nil and even lower than the bee mortality of the non-toxic standard Zolone Flo for which a slight increase in mortality was observed two to three days after application. When beehives returned to the apiary no mortality was observed.

Deltamethrin EW 15 had no or limited effect on the foraging activity in the days following the treatment on both treated and refuges areas.

Furthermore, Deltamethrin EW 15 had no effect on the bee behaviour in the days following the treatment and in the month after beehives returned to the apiary.

Reserves and brood were reduced during the study in all the tunnels, which is typical of such a study. Deltamethrin EW 15 had no negative effect on the control of the colony and this at each date of assessment.

*****

Report:	**KCP 10.3.1.5/14, [redacted] (2005)**
Title:	Assessment of the short-term effects of Deltamethrin EW 15 on behaviour, foraging activity and mortality of honeybees (*Apis mellifera*) under semifield conditions (tunnel test) in winter wheat.
Document No:	M-262484-01-1 (Rep. No.: [redacted]/AM 039)
Guidelines:	French official method CEB 230
GLP:	Yes

Material and Methods:
Test item: Deltamethrin EW 15: (development No.: 00-00308474, article No.: 00-05946743, batch No.: AAIM00846, TOX-No.: 06988-00, content of a.s. analysed: deltamethrin: 16.24 g/L), applied in treatment groups 3 and 4 at 0.42 L/ha (6.25 g a.s./ha) at a water volume of 300 L/ha.
Toxic reference item: Dimethoate EC 400 (batch No.: 37M20919, content of a.s. analysed: dimethoate: 378.05 g/L), applied at 1 L product/ha at a water volume of 300 L/ha in treatment group 2.
Treatment group 1 served as tap water treated control. In treatment group 3 the application was carried out during foraging activity of the bees, while the application in treatment group 4 was carried out in the evening, with no foraging activity. In this semifield study one replicate, represented by one tunnel (160 m²) with one bee colony (approx. 20,000 honeybees) was set up for each treatment group. Each tunnel was divided into 4 subplots (T1 to T4) of winter wheat (size of sub-plot: 10 m² each). In all treatment groups the subplots T1 to T4 received application.
The applications were performed in all 4 treatment groups after 3 days of a stable level of daily mortality. Mortality was assessed every day during 14 days (from day 6 before until day 4 after application). Foraging activity and bee behaviour were assessed every day during 10 days (from day 5 before until day 4 after application). Two evaluation checks on the weight of the colonies, the development of food stores, brood and egg laying activity of the colonies were done, one carried out at the beginning of the study (6 days before the application) and one at the end of study (6 days after the application). Additionally the number of adult bees on the combs was estimated 6 days before and 6 days after the application. Sugar solution (sugar : water 50:50) was applied every morning before the beginning of foraging activity of the honey bees from the day of hive installation in the tunnels (day 5 before the application) until day 4 after the application, at a volume of approx. 500 L/ha, to ensure the foraging activity of the bees.

Findings:
For all applications performed during the activity of bees on the crop (groups 1, 2 and 3), a sufficient number of bees was present on the crop and was exposed to the application (between 3 and 6 bees/m²). In treatment group 3 (with bees active during the application), no increase in mortality was observed at the day of treatment, and the days after. In treatment group 4, where bees were not exposed to the treatment, mortality on the day after the application was slightly higher than on the previous days but was still in the same range compared to the mortality in the control group during the whole study duration. Foraging activity in both treatment groups 3 and 4, with and without bees active during the application, decreased slightly after the application compared to the control, indicating a moderate repellent effect of the test item. Hive weight development was within the same order of magnitude in all treatment groups except in the toxic reference group where the weight decreased during the study, which can mainly be explained by the very low foraging activity during the second part of the study. The estimated number of adult bees on the combs fluctuated within a great range of variation, which is typical for this endpoint. However, a treatment-related development was not observed in this endpoint. Bee brood development and food stores were not affected by the application in the treatment groups 3 and 4. A strong decrease of brood was observed in all treatment groups, and a progression of food

stores was also observed in the same range for all treatment groups (in a lesser extent for toxic reference group).

Conclusion:
No noticeable increase of mortality was observed after the application in the tunnel where bees were directly exposed during application (treatment group 3) and in treatment group 4 (without bees present during the application). Cumulative mortality at the end of the study was comparable between the control and the treatment group without bees being present during the application, while cumulative mortality was even lower in the treatment group where bees were present during the application. Thus, no indication of a treatment-related effect was found on mortality. No treatment-related effect on colony strength was found either. A slight repellent effect of the product was observed on the day +1 and +2 after the application. The repellence was present at a greater extent in the tunnel where bees were directly exposed to the treatment. Foraging activity returned to a normal level after 3 days in both treatment groups. Likewise, other endpoints, which could pose a risk to the viability of bee colonies, were not negatively affected by the treatment. The decrease of the brood quantity during the study was observed in the same range for all treatment groups, including the control group and can be explained by the caged conditions on a cereal crop which is detrimental to bee colony development.
The application of Deltamethrin EW 15 at a rate of 0.42 L/ha (corresponding to 6.25 g a.s./ha) to cereals does not pose a risk to honeybees foraging during the application of the product.

*****

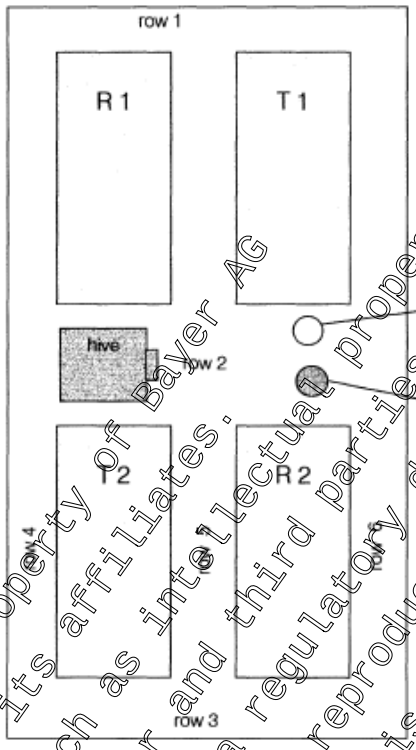
Report:	**KCP 10.3.1.5/15, [redacted]; 2001**
Title:	Impact on honeybees of Decis 100 EC insectproof tunnels on winter wheat
Document No:	M-201580-01-1 (Rep. No.: 33-2001)
Guidelines:	CEB 129
GLP:	yes

~~**Material and methods:**~~
~~Honey bee colonies (ca 18,000 to 20,000 bees per hive, colonies of comparable development, brood, and food store status, queens of homogeneous maternal origin) were confined in tunnels on winter wheat fields sprayed with sugar syrup. Two replicates were set up for the treatment, one for each control and standard. Six days after introduction of the bees into the tunnels, the application was performed. The test substance was applied at a rate of 0.0625 L/ha (corresponding to 6.25 g a.s./ha), the toxic standard was Zolone Flo (500 g/L phosalone) at a rate of 1.2 L/ha while bees were foraging. The control was treated with water. The observed endpoints were foraging activity, behaviour, mortality, and colony development.~~

~~**Findings:**~~
~~Behaviour of the bees was only slightly affected by the test item. Foraging activity was slightly influenced by the test substance only for a short time. Mortality was not increased significantly by the test item; there was a slight and short-term increase of mortality after application, but overall mortality was comparable between treatment and control. The toxic standard, however, led to a longer-lasting increase of mortality. Colony development was not affected by the treatment.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EC 100 G (AB F032640 00 EC11 A313) content of a.s.: deltamethrin: 10.6 % w/w (100 g a.s./L nominal), density: 0.958 g/ml
Batch number:	TA066/OOPM
Reference item:	Zolone Flo (active ingredient: phosalone, 500 g a.s./L nominal, analysed content: 499 g/L)
Test organism:	Honey bees (*Apis mellifera*) Bee colonies came from the same apiary containing over 1 000 hives allowing easy selection of swarms. Among the hives considered, five were chosen according to their homogeneity during the weeks preceding the test. Then four of the hives were introduced in the tunnels. Young colonies with queens from the local black breed which were one year old. These queens have a common genetic identity, they were sisters (or at least half-sisters) coming from a single strain. The colonies settled in hives of the DADANT 10 frame model. Populations spread over 7 to 8 frames (with approximately 2 to 4 frames of brood) were estimated at around 18,000 to 20,000 bees per hive.
Source:	Not reported.
Crop:	Winter wheat crop *(Triticum aestivum)*, variety: Soissons
Test location:	[redacted] on a field [redacted] France
Test unit:	Each tunnel had a half-moon support made from galvanised steel. The surface per unit was 140 m^2 (7 m x 20 m) and their roof height approximately 3 metres. A polyethylene mesh net (1.2 mm x 1.2 mm) covered the supports. Both ends were made up of the same material. Access was possible through a zip opening. Inside the tunnels, the wheat crop was split into four plots. Each had a surface of 16 m^2 (2 m x 8 m). Two plots were considered as sheltered areas (R1 and R2; not treated with test item), the other two (T1 and T2) other as treated areas. A beehive, a watering place and feeders with pollen were placed in each of the tunnels and supplied daily. Exact interior design of the tunnel is shown in the figure below:



R1/R2: sheltered area
T1/ T2: treated area

Application rates: Control (C): water
Treatment rate 1: 0.0625 L a.s./ ha (6.25 g a.s./ha) during foraging
Treatment rate 2: 0.0625 L a.s./ ha (6.25 g a.s./ha) during foraging
Reference rate (R): 1.2 L a.s./ ha (600 g a.s./ha)
Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).
The spray volume was 300-315 L/ha in all treatment groups. The sprayer was calibrated before use.

Data sampling: Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.

Data analysis: All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.

Deviations from the study plan: No deviation mentioned.

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperatures favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table.

Table 1: Weather data

	Minimum Temperature (°C)	Maximum Temperature (°C)	Rainfall (mm)
June 21st 2001	9	28	0
June 22nd 2001	10	28	0
June 23rd 2001	10	32	0
June 24th 2001	13	32	0
June 25th 2001	16	34	0
June 26th 2001	18	29	0
June 27th 2001	15	26	0
June 28th 2001	10	25	0
June 29th 2001	8	33	0
June 30th 2001	12	27	0
July 1st 2001	12	26	0
July 2nd 2001	14	30	6
July 3rd 2001	15	34	0
July 4th 2001	14	20	1
July 5th 2001	14	23	0

N.B. : Temperatures are given for the day mentioned, whereas rainfall recorded every morning shows precipitation over the previous 24 hours.

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 2: Wheat crop data

Date	Operation	Characteristics
February 21th 2001	Sowing	specy Triticum Aestivum Variety Soissons (Vérneuil seeds), dose : 200 kg / ha Batch n° F0307H00049C A sampled 10/00 Certified seeds under n° 551936 DK Treated seeds "Jockey plusab" (fluquiconazole + prochloraze-Cu + anthraquinone).
February 21th 2001	Fertiliser	Ammonitrate 33.5 % , dose : 30 u. N / ha
April 3th 2001	Fertiliser	Ammonitrate 33.5 % , dose : 50 u. N /ha
April 19th 2001	Herbicide	Allié Express (DuPont), dose : 50g / ha (carfentrazone-ethyle + metsulfuron méthyl)

The effects of Deltamethrin EW 100 G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the guidance document C.E.B. method no. 129.

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EC 100 on the honeybee, *Apis mellifera* under forced exposure conditions.

The hives were introduced into the tunnels six days prior to product application, in order to await a mortality decrease and stabilisation. The colonies were comparable to each other during our first visit at the beginning of the test period, and mortality was homogeneous the first day of the study.

Mortality in each tunnel was recorded on a daily basis for all areas covered with plastic film, from days 5DBT to 8DAT. Moreover, the day on which product application is carried out (day 0) additional counts are done at the end of the day (0DAT) in order to establish possible brutal intoxication of foraging bees. The total mortality rate recorded in a tunnel for a given day results from adding up mortality rates observed in each of the six plastic lanes in the tunnel.

Foraging was generally observed three times a day, whenever possible, in all the sheltered (R1 and R2) and treated areas (T1 and T2). It is possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts could be shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall... etc.). This parameter was also taken into account for an additional count on the day of treatment, during one hour following product application.

Two apiarist visits were programmed in the beginning and at the end of experimentation. Thus, it allowed evaluating colony development taking into account parameters: the adult bee population, the quantity and quality of the brood (different stages observed), amount of reserves and potential construction of new frames on offered wax sheets. These visits were carried out in the tunnels at dates which were as close as possible to the first and last day of confinement. However, for practical or climatic conditions, they necessarily took place within 48 hours on the one hand before or after introduction of the hives in the tunnels, and on the other hand when the hives were taken out.

Assessments of bee behaviour were carried out when products were applied and during the thirty minutes following product application. In general, this observation phase continued all over the day between counts. Bees were especially observed for reactions and behaviour like intense flying, bee clusters on the net or at the entrance of the hive, aggressiveness, beginning of an intoxication etc. in each of the tunnels.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Number of foraging bees/m^2 per day on all the areas (T1, T2 and R1, R2) before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the test item groups and the reference item group, respectively
- Colony Assessment in the beginning and at the end of experimentation

Dates of Work: 21th June to 5th July 2001

Findings:

Honey bee mortality

A summary of the daily mortality and total mortality results are shown in the following table.

Table 3: Daily mortality data

Treatment	5DBT - 22 June						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	67	34	46	55	8	16	**226**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	69	12	40	25	6	16	**168**
Water control	82	11	43	40	6	38	**220**
Zolone Flo	91	15	62	17	6	73	**264**
	4DBT - 23 June						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	31	5	25	27	5	16	**109**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	35	12	32	11	2	15	**107**
Water control	54	9	37	15	3	9	**127**
Zolone Flo	52	12	32	10	4	29	**139**
	3DBT - 24 June						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	23	2	43	12	0	7	**87**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	28	7	27	11	4	18	**95**
Water control	17	16	31	8	1	22	**95**
Zolone Flo	56	10	44	12	5	21	**148**
	2DBT - 25 June						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	52	10	58	28	5	5	**158**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	22	15	27	12	6	16	**98**
Water control	52	9	48	23	3	21	**156**
Zolone Flo	93	17	74	33	3	38	**258**
	1DBT - 26 June						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	26	4	68	21	2	3	**124**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	16	11	37	11	3	8	**86**
Water control	23	16	80	24	1	8	**152**
Zolone Flo	87	48	132	22	3	57	**349**
	0DBT - 27 June morning						
zone	lane 1	lane 2	lane 3	lane 4	lane 5	lane 6	total

Deltamethrin EC 100 (1) at 6.25 g a.s./ha	53	8	37	15	1	9	**123**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	88	11	9	7	2	9	**126**
Water control	119	10	44	16	5	21	**215**
Zolone Flo	163	38	48	26	15	172	**462**
	0DAT - 27 June afternoon						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	103	9	23	18	1	9	**163**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	95	9	11	2	2	10	**129**
Water control	77	5	5	3	2	10	**102**
Zolone Flo	510	29	49	35	13	138	**774**
	1DAT - 28 June						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	103	9	23	18	1	9	**163**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	95	9	11	2	2	10	**129**
Water control	77	5	5	3	2	10	**102**
Zolone Flo	510	29	49	35	13	138	**774**
	2DAT - 29 June						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	112	4	76	14	4	9	**219**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	91	7	16	6	1	25	**146**
Water control	138	5	24	8	1	14	**190**
Zolone Flo	932	113	117	68	28	303	**1561**
	3DAT - 30 June						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	53	3	56	32	0	9	**153**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	69	6	18	10	1	9	**114**
Water control	71	3	50	26	2	23	**175**
Zolone Flo	363	71	169	96	11	229	**939**
	4DAT – 1 July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	124	3	65	24	3	18	**234**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	136	9	19	10	3	13	**190**
Water control	124	5	33	19	2	11	**194**
Zolone Flo	499	56	96	73	8	128	**860**
	5DAT - 2 July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	64	12	47	41	5	3	**172**

Deltamethrin EC 100 (2) at 6.25 g a.s./ha	80	9	47	37	6	14	**193**
Water control	76	9	50	15	1	13	**164**
Zolone Flo	300	40	161	85	15	119	**720**
	6DAT - 3 July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	54	8	109	85	9	8	**273**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	65	7	65	35	3	17	**192**
Water control	61	23	114	25	2	21	**246**
Zolone Flo	126	44	184	60	15	124	**553**
	7DAT - 4 July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	126	28	84	57	5	20	**320**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	100	17	70	40	1	21	**249**
Water control	88	21	84	37	3	20	**253**
Zolone Flo	150	43	80	34	2	73	**382**
	8DAT - 5 July						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Deltamethrin EC 100 (1) at 6.25 g a.s./ha	55	25	41	10	11	34	**176**
Deltamethrin EC 100 (2) at 6.25 g a.s./ha	59	21	22	6	9	18	**135**
Water control	43	41	23	8	17	25	**157**
Zolone Flo	99	48	40	17	4	60	**268**

DBT: days before treatment
DAT: days after treatment

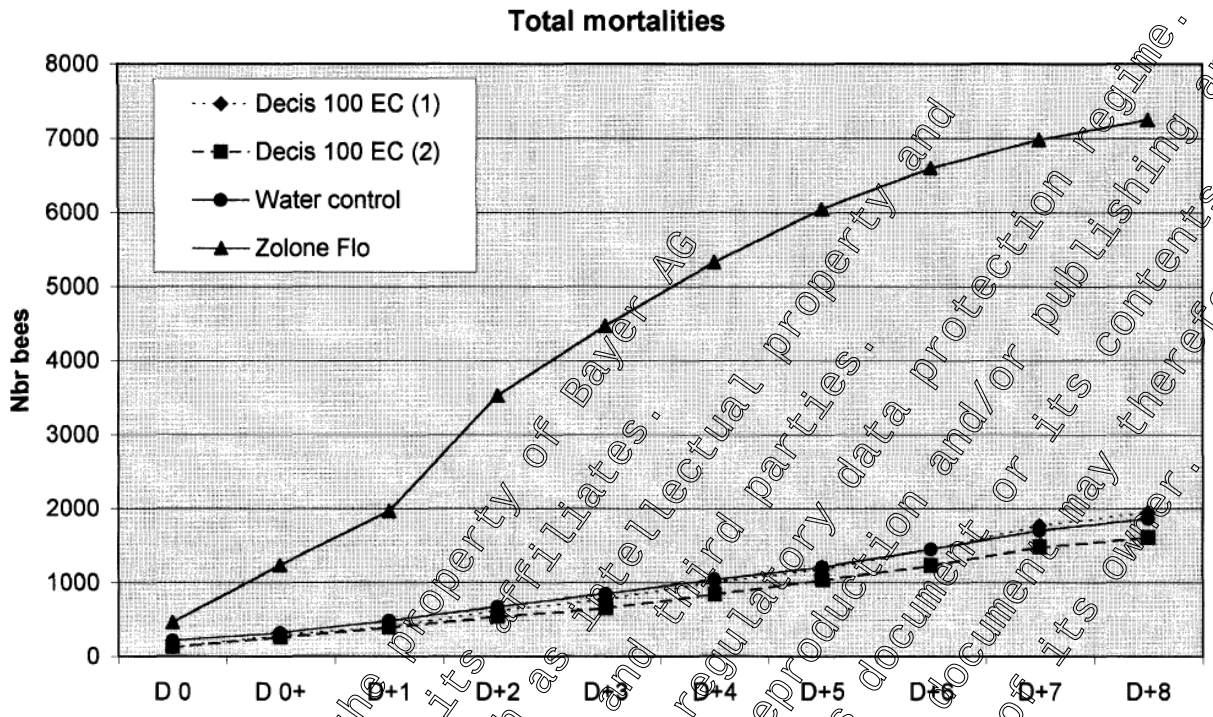


Figure 1: Total mortalities for Deltamethrin EC 100 (1) at 6.25 g a.s./ha (Decis 100 EC), Deltamethrin EC 100 (2) at 6.25 g a.s./ha (Decis 100 EC), the water control and the reference item (Zolone Flo)
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+8: 1 to 8 days after treatment

The day after the treatment, mortality trends showed real differences. A high peak of mortality occurred in the reference Zolone Flo tunnel. The elevation of mortality in both tunnels treated with Deltamethrin EC 100 showed that mortality levels at 1DAT in these tunnels stayed comparable to the untreated control. This development is linked to the treatment day only, and levels were decreasing on the next day (2DAT).

The data validation was available as Zolone Flo showed an increasing mortality whereas the water control tunnel showed a regular evolution. In this control tunnel (treated with water) the colony was not disturbed by the treatment. Mortality rates recorded varied few along the week.

After treatment the difference between Deltamethrin EC 100 formulations and Zolone Flo was linked not only to the intensity but also to the duration effect. The standard tunnel treated with phosalone showed a high mortality for several days. This level of mortality stayed high from 1DAT to 3DAT and we had to wait until 3DAT looking to a decreasing mortality, then until 7DAT to obtain similar mortality data.

The daily mortality trends in both tunnels treated with Deltamethrin EC 100 were comparable to that of the water control tunnel. There was, however, a small increase in mortality rates following product application observed only at 1DAT. This increase in mortality rates was limited in intensity and observed shortly. Until the end of the trial this parameter regularly remained in the same values as in the untreated tunnel.

Total dead bees were comparable between the Deltamethrin EC 100 tunnels and the control at the end of this experimental phase as well as on the day of application. So these trends could be considered as

close from one another, with non-high toxicity described in the tunnels where Deltamethrin EC 100 was applied.

Foraging activity

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following tables.

Table 4: Foraging data: Deltamethrin EC 100 (1) (Decis 100 EC) at 6.25 g a.s./ha

Decis 100 EC (1)		raw data - nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
June 25th 01	8H45	97	34	102	77	76	69	105	66	155	158	9,7	9,9
	9H30	114	48	87	105	88	82	112	79	177	176	11,1	11,0
D-2	10H45	141	54	95	69	69	81	112	74	180	168	11,2	10,5
											167		10,5
June 26th 01	8H00	121	64	117	73	106	84	110	87	188	194	11,7	12,1
D-1	8H45	110	65	108	69	105	81	120	74	172	180	10,7	11,3
	10H30	137	88	155	104	149	101	157	113	242	260	15,1	16,3
											211		13,2
June 27th 01	9H00	84	160	96	179	109	183	116	141	260	275	16,2	17,2
	9H30	119	168	105	160	91	158	141	144	271	267	16,9	16,7
D 0											271		16,9
	11H30	29	25	35	22	14	9	16	8	54	24	3,3	1,5
	12H00	26	12	28	21	16	5	8	1	44	47	2,7	1,8
	14H30	2	1	1	6	1	0	1	3	5	3	0,3	0,2
											14		0,9
June 28th 01	10H00	191	109	153	130	160	78	127	101	292	233	18,2	14,6
D+1	11H00	146	68	96	63	109	47	72	64	197	146	12,3	9,1
	12H00	101	57	63	46	49	22	33	25	134	65	8,3	4,0
											148		9,2
June 29th 01	10H00	245	110	178	150	174	133	172	116	342	298	21,3	18,6
D+2	11H00	168	104	148	99	99	71	105	94	256	185	16,0	11,5
	12H00	122	65	87	59	66	48	69	74	172	129	10,7	8,0
											204		12,7

R1a: number of bees counted on half of the sheltered area n°1 by a first experimenter.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimenter.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimenter.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimenter
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) /2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².

Table 5: Foraging data: Deltamethrin EC 100 (2) (Decis 100 EC) at 6.25 g a.s./ha

Decis 100 EC (2)		raw data - nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
June 25th 01	8H45	127	66	91	56	84	83	106	75	170	174	10,6	10,9
	9H30	131	69	92	63	77	83	104	82	178	173	11,1	10,8
D-2	10H45	111	82	103	71	83	81	105	88	184	179	11,5	11,2
											175		10,9
June 26th 01	8H00	125	88	112	97	96	90	114	118	211	209	13,2	13,1
D-1	8H45	84	82	91	63	74	86	92	103	160	178	10,0	11,1
	10H30	175	111	156	79	140	120	151	96	261	261	16,3	15,8
											213		13,2
June 27th 01	9H00	85	165	70	136	129	133	105	131	228	249	14,3	15,6
	9H30	123	145	90	122	140	127	104	145	240	258	15,0	16,1
D0											254		15,8
	11H30	46	23	25	14	7	6	11	7	54	16	3,4	1,0
	12H00	35	19	21	13	5	2	8	5	44	10	2,8	0,6
	14H30	11	13	14	3	6	4	2	3	21	8	1,3	0,5
											11		0,7
June 28th 01	10H00	182	85	73	67	71	68	75	69	204	142	12,7	8,9
D+1	11H00	160	112	57	53	61	42	64	48	191	108	11,9	6,7
	12H00	99	50	51	17	26	10	19	8	109	30	6,8	1,9
											93		5,8
June 29th 01	10H00	196	122	142	106	103	88	135	103	283	215	17,7	13,4
D+2	11H00	191	84	105	93	77	54	102	81	237	157	14,8	9,8
	12H00	128	82	80	71	64	38	62	42	181	103	11,3	6,4
											158		9,9

R1a: number of bees counted on half of the sheltered area n°1 by a first experimenter.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimenter.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimenter.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimenter
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16 Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².

Table 6: Foraging data: Water Control

Water control		raw data - nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
June 25th 01	8H45	71	29	107	85	62	70	136	65	146	167	9,1	10,4
	9H30	45	11	76	70	34	41	94	47	101	108	6,3	6,8
D-2	10H45	68	33	87	36	63	51	72	54	112	120	7,0	7,5
											114		7,1
June 26th 01	8H00	65	61	96	64	72	62	87	73	143	147	8,9	9,2
D-1	8H45	51	51	78	53	50	42	63	65	117	110	7,3	6,9
	10H30	131	82	92	60	126	82	93	63	183	182	11,4	11,4
											146		9,1
June 27th 01	9H00	100	167	86	100	122	134	71	113	227	220	14,2	13,8
	9H30	81	132	87	87	92	104	77	101	194	187	12,1	11,7
D0											204		12,7
	11H30	75	38	48	37	51	61	58	38	99	104	6,2	6,5
	12H00	54	41	52	34	48	37	44	30	91	80	5,7	5,0
	14H30	34	27	30	22	33	23	31	18	57	53	3,5	3,3
											79		4,9
June 28th 01	10H00	111	84	90	78	64	80	135	99	182	189	11,3	11,8
D+1	11H00	70	42	63	45	45	42	87	73	110	124	6,9	7,7
	12H00	74	43	48	42	36	32	65	45	104	84	6,5	5,3
											132		8,3
June 29th 01	10H00	87	65	88	719	75	95	108	96	180	187	11,3	11,7
D+2	11H00	54	60	69	74	55	59	97	71	129	141	8,0	8,8
	12H00	52	41	63	33	48	40	65	46	95	100	5,9	6,2
											143		8,9

R1a: number of bees counted on half of the sheltered area n°1 by a first experimenter.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimenter.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimenter.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimenter
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation: R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².

Table 7: Foraging data: Zolone Flo

Zolone Flo		raw data - nbr bees								calculated data			
day	time	R1a	R1b	R2a	R2b	T1a	T1b	T2a	T2b	mean zR	mean zT	R / m²	T / m²
June 25th 01	8H45	84	51	62	94	70	81	60	42	146	127	9,1	7,9
	9H30	38	28	26	45	32	55	37	13	69	69	4,3	4,3
D-2	10H45	72	48	33	46	78	105	95	42	100	160	6,2	10,0
											118		7,4
June 26th 01	8H00	60	49	52	38	57	57	76	49	100	120	6,2	7,5
D-1	8H45	54	34	40	36	52	46	54	45	82	99	5,1	6,2
	10H30	107	75	106	74	145	117	104	62	181	214	11,3	13,4
											144		9,0
June 27th 01	9H00	96	163	86	112	153	158	95	156	229	281	14,3	17,6
	9H30	143	164	73	71	120	87	111	122	225	220	14,1	13,8
D0											251		15,7
	11H30	113	59	31	35	84	68	63	53	119	134	7,4	8,4
	12H00	109	60	30	31	50	47	39	35	115	86	7,2	5,3
	14H30	42	45	17	21	20	25	12	13	63	35	3,9	2,2
											85		5,3
June 28th 01	10H00	80	42	50	46	51	53	33	28	109	83	6,8	5,2
D+1	11H00	38	37	13	19	20	39	13	16	54	44	3,3	2,8
	12H00	30	24	23	23	22	23	4	4	50	27	3,1	1,7
											51		3,2
June 29th 01	10H00	67	55	68	62	66	51	26	25	126	81	7,9	5,1
D+2	11H00	47	37	32	35	28	42	16	15	76	51	4,7	3,2
	12H00	43	24	33	22	31	47	15	10	61	52	3,8	3,2
											61		3,8

R1a: number of bees counted on half of the sheltered area n°1 by a first experimenter.
R1b: number of bees counted on the other half of the sheltered area n°1 by a second experimenter.
R1t: total number of bees counted on the whole of the sheltered area n°1.
T1a: number of bees counted on half of the treated area n°1 by a first experimenter.
T1b : number of bees counted on the other half of the treated area n°1 by a second experimenter
T1t: total number of bees counted on the whole of treated area n°1.
Counts are expressed in the same way for areas R2 and T2.
Nb bees/m² : number of mean bees per square meter on R (sheltered area) or T (treated area). Precision calculation:
R = [(R1t + R2t) / 2] /16. Same for calculating T.
Mean zt: number of mean bees on a treated area of 16 m².

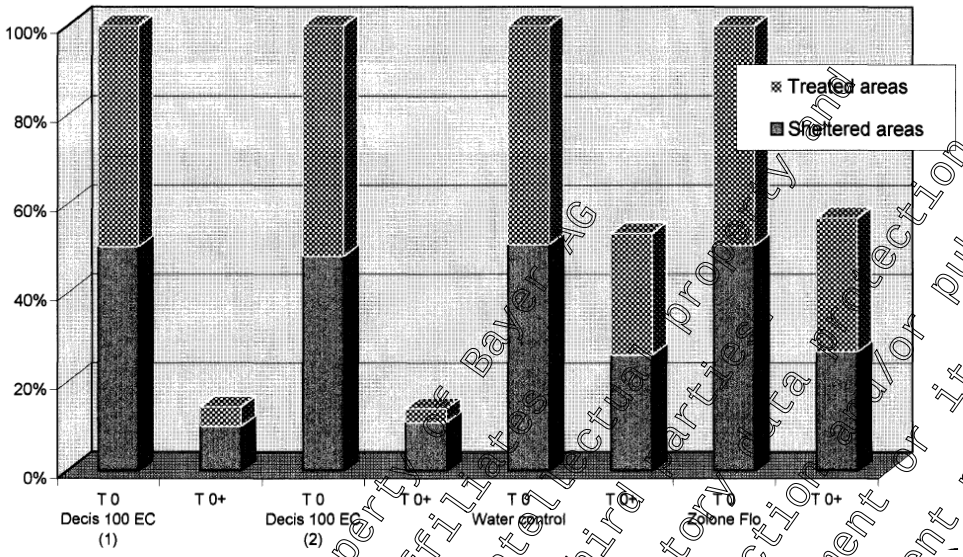


Figure 2: Breakdown in foraging on treatment day in treated and sheltered areas for Deltamethrin EC 100 (1) at 6.25 g a.s./ha (Decis 100 EC), Deltamethrin EC 100 (2) at 6.25 g a.s./ha (Decis 100 EC), the water control and the reference item (Zolone Flo)
T0: before product application
T0+: after product application

During the three counts that followed product application, mean foraging trends were comparable between tunnels. In all tunnels this activity decreased a lot during the afternoon. Not only the impact of substances could be involved, as foraging decreased in the untreated tunnel too. In fact this activity was always more intensive in the morning in reason of sugar spraying. Then it decreased as climatic conditions induced evaporation and crystallisation of the syrup. On this special day (day 0) it was impossible to spray more water on the plots in order to keep them attractive. So most bees left the crop for the end of the day in all tunnels.

The main decrease appeared in both Deltamethrin EC 100 tunnels where the foraging activity was close to null in the afternoon. Less important was the decrease of activity in the control tunnel, as well as in the reference tunnel where bees get slowly intoxicated despite a characteristic smell.

On the following days foragers' activity increased with different intensity. It increased regularly for two days in Deltamethrin EC 100 formulation tunnels, while it remained close to stable in the untreated tunnel at 1DAT and 2DAT. In these three tunnels activity levels reached one another at this time. On the contrary foraging activity remained lower in the Zolone Flo tunnel where crop treated plots were less attractive to bees.

Shortly after product application (0DAT, during the thirty minutes following product application), a repulsive effect was observed in both Deltamethrin EC 100 tunnels. The decrease in foraging activity affected all tunnel and mostly treated areas.

Obviously spraying disturbed the bees, as foraging activity decreased in control tunnel too. In this water control tunnel, as well as in the reference Zolone Flo tunnel there was no repulsive effect, the decrease of activity was similar on all treated and sheltered areas.

During spraying experimentators experienced neither particular aggressiveness nor any frenetic bumbling. In the tunnels where the test product was applied, a few clinical signs occurred in the hour

following product application. These signs were observed in the afternoon but not the next or following days.

Intoxication symptoms: Description had to be compared to what happened in the standard tunnel. Foragers in contact with the product were the ones that were affected first. In Deltamethrin EC 100 and Zolone Flo tunnels, some bees were on the ground after treatment and they had typical intoxication signs.

In these tunnels, intoxicated bees fell on the plastic surface of the rows, walked in a difficult way alternating periods of sluggishness and frenzy. Such a bee rolled over itself and appeared too heavy when trying to lift off. It's fore legs then its hind legs and abdomen appeared to be paralysed. The bee died in a range from a few minutes to a few hours.

Behaviour of the bees

Colony behaviour was comparable between tunnels as foraging was quite regular on crop plots. Colonies in the different tunnels only showed reaction to treatments as flying away when the boom with water passes by.

In the standard tunnel, a characteristic Zolone Flo smell appeared after treatment and remains for several hours. A few intoxication signs also appeared and are more frequent by the end of the day, either on the next days (1DAT to 4DAT).

Activity at the hive entrance was normal in all tunnels. No bee clusters were observed either on the nets or at the hive entrance and no fleeing events were observed in any of the tunnels.

Intoxication symptoms: Description had to be compared to what happened in the standard tunnel. Foragers in contact with the product were the ones that were affected first. In Deltamethrin EC 100 and Zolone Flo tunnels, some bees were on the ground after treatment and they have typical intoxication signs.

In these tunnels, intoxicated bees fall on the plastic surface of the rows, walk in a difficult way alternating periods of sluggishness and frenzy. Such a bee rolls over itself and appears too heavy when trying to lift off. It's fore legs then its hind legs and abdomen appears to be paralysed. The bee died in a range from a few minutes to a few hours.

Conclusion:

Overall conditions for conducting the experimental phase of the trial were favourable to beekeeping. Climatic and crop conditions were satisfactory so that the different parameters observed agree with the data obtained.

According to this experimental phase, especially reference Zolone Flo showed an impact on bee death compared to the other tunnels. A main observation was observed in mortality caused by Zolone Flo. This substance showed high effects over several days, when Deltamethrin EC 100 effect is limited to a very small increase of mortality, only the following day after application.

In both two tunnels where Deltamethrin EC 100 was used, mortality level reaches water control data the day after treatment (1DAT).

Experimental conditions of the study were quite strict, including confinement and product application carried out during foraging activity, on attractive plots. Only the use of Zolone Flo gave a mortality stage that remained a few days.

In the case of this trial under tunnels on a wheat crop, the effects of the test substance Deltamethrin EC 100 in two tunnels only showed a low and temporary increase in mortality yielding comparable total mortality rates to those recorded in the water control tunnel.

In these tunnels (except reference Zolone Flo) evolution of mortality was similar all along the trial. This was illustrated as curves were close together on the graphs.

****

Report:	**KCP 10.3.1.5/16, [redacted], 2006**
Title:	Evaluation of effects on honey bees of one Deltamethrine 100 EC application on winter wheat
Document No:	M-268997-01-1 (Rep. No.: 87-2005)
Guidelines:	CEB 230, EPPO 170
GLP:	yes

~~**Material and methods:**~~

~~Honey bee colonies (ca 15,000 to 20,000 bees per hive, colonies of comparable development, brood, and food store status, queens of homogeneous maternal origin) were confined in tunnels on winter wheat fields sprayed with sugar syrup. One replicate was set up for the treatment and one for each control and standard. Deltamethrin 100 EC is applied in two modalities, during the bee foraging activity under one tunnel, and at night in another tunnel in order to avoid the contact with forager bees. The test substance was applied at a rate of 0.0625 L/ha (= 6.25 g a.s./ha), the toxic standard was Dimezyl 40 EC (40 g/L dimethoate) at a rate of 1.0 L/ha while bees were foraging. The control was treated with water. The observed endpoints were foraging activity, behaviour, mortality, and colony development.~~

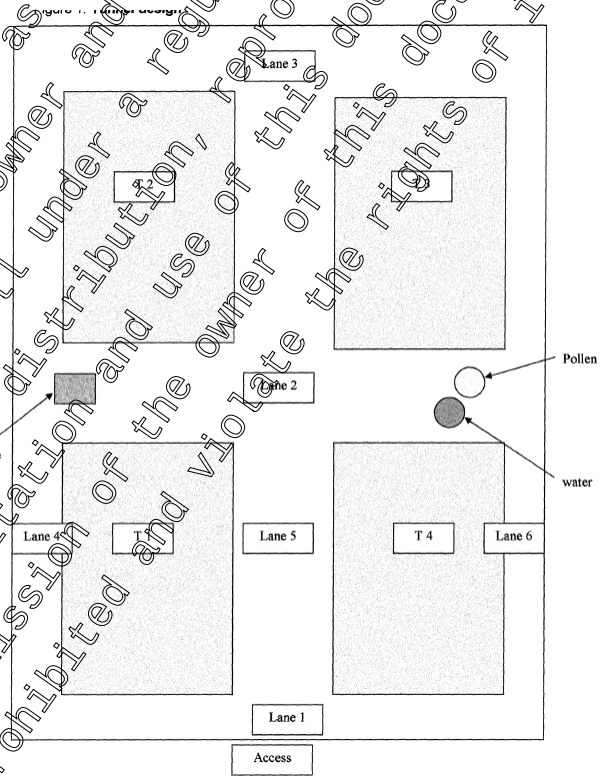
~~**Findings:**~~

~~Behaviour of the bees was only slightly affected by the test item. Foraging activity was slightly influenced by the test substance only for a short time. Mortality was not increased significantly by the test item; there was a slight and short term increase of mortality after application, but overall mortality was comparable between treatment and control. The toxic standard, however, led to a longer lasting increase of mortality. Colony development was not affected by the treatment.~~

Material and Methods:

Test material:	Deltamethrin
Test item:	Deltamethrin EC 100 G (AE F032640 00 EC11 A3) content of a.s.: deltamethrin: 102.08 g a.s./L (100 g a.s./L nominal), density: 0.954 g/ml
Batch number:	OP240841
Reference item:	Dimezyl (active ingredient: dimethoate, 400 g a.s./L nominal, analysed content: 400.9 g/L)
Test organism:	Honey bees (*Apis mellifera*) Bee colonies were especially selected over 1000 hives that permits a selection of swarms. Queens in the four colonies were one year

	old. As they come from a single strain they had a common genetic identity. More, they were sisters (or half-sisters) from the local black breed. These colonies were settled in small hives of the DADANT 10 frames model. Populations spread over the six frames (of which approximately 3 to 5 frames of brood) were estimated at around 15,000 to 20,000 bees per hive.
Source:	Not reported.
Crop:	Winter wheat crop during BBCH main stage n°5, variety: Apache
Test location:	[redacted] on a field [redacted] France
Test unit:	Each tunnel had a half-moon support made from galvanised steel; the hoops were nailed in the soil and joined with crossbars. The surface per unit was 140 m^2 (7 m x 20 m) and their roof height approximately 3 metres. A polyethylene mesh net (1.2 mm x 1.2 mm) covered the supports. Both ends are made up of the same material. Access is possible through a zip opening. Exact interior design of the tunnel is shown in the figure below:



Application rates:	Control (C): water Treatment rate 1: 0.0625 L a.s./ ha (6.25 g a.s./ha) during foraging activity Treatment rate 2: 0.0625 L a.s./ ha (6.25 g a.s./ha) out of foraging activity Reference rate (R): 1 L/ ha (400 g a.s./L) Every treatment comprised of one replicate (i.e. 1 tunnel per treatment). The boom was previously tested on a calibration scale in order to check the homogeneity level of the nozzle flow.
Data sampling:	Data for mortality, foraging activity and data of the colony were assessed.
Data analysis:	All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.
Deviations from the study plan:	No deviation mentioned.

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperatures favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table.

Table 1: Weather data

	Temperature Min (°c)	Temperature Maxi (°C)	Rainfall (mm)
May 16th 2005	13	27	0
May 17th 2005	13	29	0
May 18th 2005	18	31	0
May 19th 2005	17	28	0
May 20th 2005	16	30	0
May 21st 2005	18	31	2
May 22nd 2005	15	24	5
May 23rd 2005	20	37	0
May 24th 2005	16	29	0
May 25th 2005	17	27	0

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 13: Winter wheat crop data

Date	Operation	Characteristics
October 2004	Soil preparation	Harrowing: preparation of the seed bed and adventice destruction
October 29th 2004	Sowing	Specie : triticum Variety : Apache sample: F0389M030203 treatment: Celest (fodioxonil + anthraquinone) sowing dose: 180 kg/ha
February 9th 2005	Fertiliser	Ammonitrate : 80 u N / ha
May 25th 2005	Destruction	Harrowing with cover crop

The effects of Deltamethrin EW 15 G were tested on the honeybee (*Apis mellifera* L.) under confined semi-field conditions by following the guidance document C.E.B. method no. 230.

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EC 100 G on the honeybee, *Apis mellifera* under forced exposure conditions.

Four tunnels were built up for this trial. Deltamethrin 100 EC was applied in two modalities, during the bee foraging activity under one tunnel, and at night in another tunnel in order to avoid the contact with forager bees. In the same foraging conditions, a toxic reference with dimethoate (400g a.i./ha) and a water control was applied for comparisons.

Colonies were introduced into the tunnels on Sunday May 15th 2005 at night. The colonies were comparable to each other during our first visit at the beginning of the test period, they look homogeneous during these days. After a few days of confinement, foraging bees' activity was adapted to the considered area. Daily mortalities were collected all over plastic covered lanes.

Mortality in each tunnel was recorded on a daily basis for all areas covered with plastic film, from days 2DBT to 6DAT. The total mortality rate recorded in a tunnel for a given day resulted from adding up mortality rates observed in each of the six plastic lanes in the tunnel.

The quantity of forager bees was observed during seven days from 3DBT to 3DAT, on all the crop plots. It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts can be shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall...etc.).

Two apiarist visits was programmed in the beginning and at the end of experimentation, allowing evaluating colony development. Parameters taking into account was the adult bee population, the quantity and quality of the brood (different stages observed), and amount of reserves. These visits were carried out in the tunnels at dates which were as close as possible to the first and last day of confinement.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Number of foraging bees/m^2 per day on all the areas before as well as after the applications in the control, the test item groups and the reference item group, respectively
- Colony Assessment in the beginning and at the end of experimentation

Dates of Work: 16th May to 25th May 2005

Findings:

Honey bee mortality

A summary of the daily mortality and total mortality results are shown in the following table.

Table 14: Daily mortality data

Treatment	2DBT – 17 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	30	16	18	36	4	12	**126**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	22	17	38	23	1	9	**110**
Dimethoate	105	32	234	123	5	35	**534**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	54	45	101	48	16	30	**294**
	1DBT - 18 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	56	20	34	45	2	6	**163**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	56	16	49	36	6	23	**186**
Dimethoate	174	18	168	126	10	36	**532**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	82	60	71	67	14	36	**330**
	0DBT – 19 May morning						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	39	11	46	17	2	18	**133**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	58	7	63	16	3	18	**165**
Dimethoate	183	28	175	44	10	70	**510**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	78	16	98	40	18	34	**284**
	0DBT – 19 May afternoon						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	21	5	12	7	0	13	**58**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	/	/	/	/	/	/	**0**

Dimethoate	226	339	232	144	17	430	**1388**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	74	13	36	9	9	36	**177**
	1DAT – 20 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	20	7	29	9	2	10	**77**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	60	7	60	10	3	37	**177**
Dimethoate	505	7	415	311	36	583	**3187**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	65	24	95	17	9	45	**255**
	2DAT - 21 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	88	5	25	22	1	22	**169**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	178	8	37	18	5	68	**314**
Dimethoate	1016	951	111	258	56	394	**2786**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	142	10	33	9	12	46	**248**
	3DAT - 22 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	65	8	43	14	7	42	**179**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	51	5	33	8	2	36	**135**
Dimethoate	253	179	57	23	11	204	**727**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	84	17	47	12	10	43	**213**
	4DAT - 23 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	62	7	28	5	1	40	**143**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	52	11	19	4	8	24	**118**
Dimethoate	166	160	16	11	10	117	**480**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	79	10	17	11	5	27	**149**
	5DAT – 24 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	165	8	34	28	13	31	**279**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	106	9	21	19	6	38	**199**
Dimethoate	440	73	24	21	7	85	**650**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	149	16	27	11	6	40	**249**

	6DAT – 25 May						
zone	**lane 1**	**lane 2**	**lane 3**	**lane 4**	**lane 5**	**lane 6**	**total**
Control	141	37	87	59	7	34	**365**
Deltamethrin 100 out of foraging (6.25 g a.s./ha)	88	22	73	30	5	48	**266**
Dimethoate	240	161	113	91	5	93	**703**
Deltamethrin 100 during foraging (6.25 g a.s./ha)	80	19	115	37	24	67	**382**

DBT: days before treatment
DAT: days after treatment

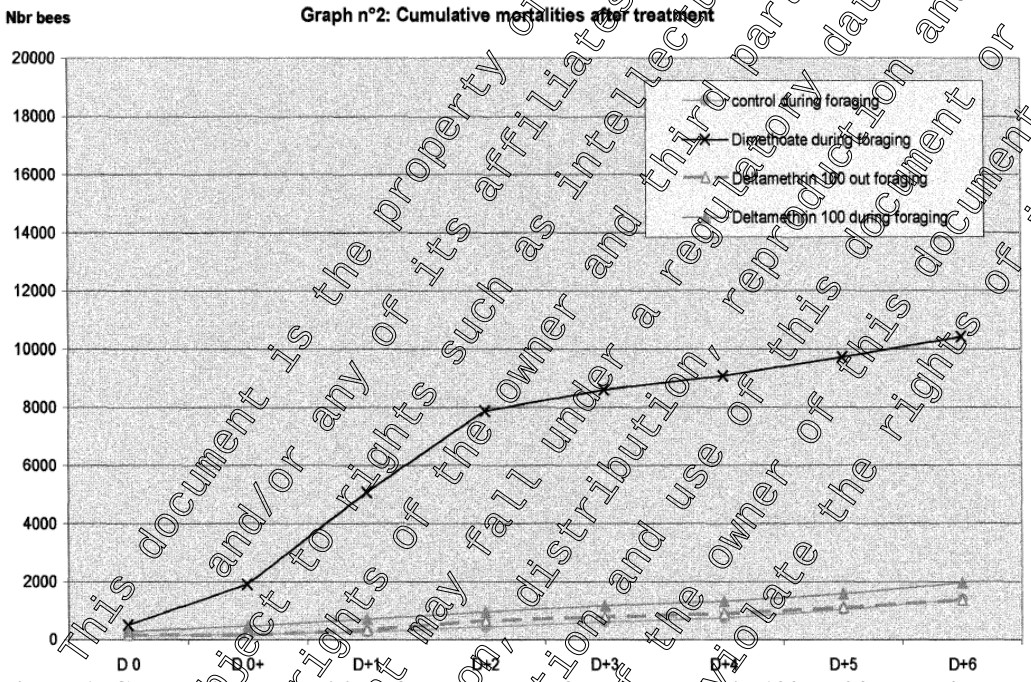


Figure 1: Cumulative mortalities after treatment for Deltamethrin 100 at 6.25 g a.s./ha during foraging, Deltamethrin 100 at 6.25 g a.s./ha out of foraging, for the control and the reference item (Dimethoate)
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+6: 1 to 6 days after treatment

The main information as a result according to the use of Deltamethrin 100 EC was the similarity of the mortality development within the two treatments (during and out of foraging). Whatever the application time, in presence or in absence of forager bees on the wheat plots, this Deltamethrin 100 EC induced similar records according to the daily mortalities. Application during foraging of bees its impact led to a limited increase of mortality that could be hardly observed only on the following day (1DAT). This development was limited to this following day, as then the mortality trends in both treatments were similar until the end of the recording period. Both mortality levels from 2DAT to 5DAT were low and comparable to their previous levels before applications.

In these experimental conditions Deltamethrin 100 EC (0.0625 L/ha) induces no peak of mortality. More, when applied out of the bee presence on the plots, mortality trend was comparable to the water control and curves similar on a graph. In these experimental conditions Deltamethrin 100 EC

(0.0625 L/ha) applied at night when no honeybee forages had no impact on the mortality.

On another view, the graph "Cumulative mortalities after treatment" expresses the effect of the different items from the treatment day to 6DAT, in order to look at cumulative impact after treatment. Curves on this graph revealed the impact of dimethoate as the highest of all items in this trial (over 10 000 bees). The formulation Deltamethrin 100 EC (0.0625 L/ha) provided low numbers of total dead bees after six days whatever the time of application. This formulation seemed to be very safe to bees in these experimental conditions.

Foraging activity

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following tables.

Table 15: Foraging data: Water control

	raw data / nbr of bees								data calculated	
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 16th 2005	16:00	31	18	19	13	26	12	40	32	48	3,0
D-3										48	3,0

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 17th 2005	12:20	4	10	6	11	14	12	28	25	28	1,8
D-2	15:10	20	18	6	7	17	12	27	29	34	2,1
										31	1,9

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 18th 2005	12:15	35	14	25	27	21	24	28	18	49	3,0
D-1										49	3,0

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
	10:15	67	46	50	52	75	70	47	55	124	7,7
Day	14:15	48	14	44	27	50	36	45	32	74	4,6
May 19th 2005										99	6,2
D 0	15:55	13	11	20	39	36	29	38	23	52	3,3
	16:45	20	4	28	24	48	37	43	38	61	3,8
										56	3,5

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 20th 2005	09:40	65	120	91	121	103	145	115	121	220	13,8
D+1										220	13,8

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 21st 2005	13:30	130	54	145	91	126	95	96	104	210	13,1
D+2	15:00	109	54	62	48	66	57	65	53	128	8,0
										169	10,6

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 22nd 2005	13:00	109	45	81	65	71	58	65	69	141	8,8
D+3										141	8,8

T : number of bees counted on the treated area.
T/ m² : number of mean bees per square meter
mean zT : number of mean bees per crop plot
D-3 to D-1 : 3 to 1 days before application
DO: 0 days before application
D+1 to D+3: 1 to 3days after application

Table 16: Foraging data: Deltamethrin 100 out of foraging at 6.25 g a.s./ha

		raw data / nbr of bees								data calculated	
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 16th 2005	16:00	39	31	34	29	31	19	60	13	64	4,0
D-3										64	4,0
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 17th 2005	12:20	18	25	20	30	26	24	33	29	51	3,2
D-2	15:10	16	38	19	40	17	17	23	42	53	3,3
										52	3,3
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 18th 2005	12:15	24	17	35	16	19	25	21	29	47	2,9
D-1										47	2,9
	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
	10:15	73	76	90	92	116	105	62	96	178	11,1
Day	14:15	64	41	95	35	54	43	196	57	124	7,7
May 19th 2005										151	9,4
D 0	15:55	28	32	40	35	47	21	35	31	67	4,2
	16:45	25	31	57	37	55	46	51	32	82	5,1
										75	4,7
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 20th 2005	09:40	33	32	29	35	34	31	37	32	66	4,1
D+1										66	4,1
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 21st 2005	13:30	79	84	93	56	58	74	70	73	146	9,1
D+2	15:00	58	56	60	90	75	42	58	73	128	8,0
										137	8,6
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 22nd 2005	13:00	58	32	43	37	40	39	47	54	90	5,6
D+3											

T : number of bees counted on the treated area
T/ m^2 : number of mean bees per square meter
mean zT: number of mean bees per crop plot
D-3 to D-1: 3 to 1 days before application
DO: 0 days before application
D+1 to D+3: 1 to 3days after application

Table 17: Foraging data: Dimethoate during foraging

	raw data / nbr of bees								data calculated	
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 16th 2005	16:00	46	52	48	27	18	36	49	117	98	6,1
D-3										98	6,1
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 17th 2005	12:20	52	46	23	63	57	19	46	82	97	6,1
D-2	15:10	63	40	49	19	47	56	58	43	94	5,9
										95	6,0
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 18th 2005	12:15	46	28	52	37	56	38	48	53	90	5,6
D-1										90	5,6
	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
	10:15	57	42	68	51	33	67	49	57	106	6,6
Day	14:15	135	93	142	72	92	147	124	113	230	14,3
May 19th 2005										168	10,5
D 0	15:55	10	4	6	4	5	6	2	5	11	0,7
	16:45	2	0	3	2	1	0	0	0	2	0,1
										6	0,4
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 20th 2005	09:40	0	0	0	0	0	1	0	0	0	0,0
D+1										0	0,0
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 21st 2005	13:30	0	0	0	0	0	0	0	0	0	0,0
D+2	15:00	0	0	0	0	0	0	0	0	0	0,0
										0	0,0
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 22nd 2005	13:00	0	0	0	0	0	0	0	0	0	0,0
D+3											

T : number of bees counted on the treated area
T/ m^2 : number of mean bees per square meter
mean zT: number of mean bees per crop plot
D-3 to D-1: 3 to 1 days before application
DO: 0 days before application
D+1 to D+3: 1 to 3days after application

Table 18: Foraging data: Deltamethrin 100 during foraging at 6.25 g a.s./ha

	raw data / nbr of bees									data calculated	
Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 16th 2005	16:00	40	40	41	21	36	32	48	22	70	4,4
D-3										70	4,4

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 17th 2005	12:20	13	41	19	40	33	26	36	39	62	3,9
D-2	15:10	29	48	22	42	23	28	49	36	69	4,3
										66	4,2

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 18th 2005	12:15	12	8	24	17	17	16	15	14	31	1,9
D-1										31	1,9

	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
	10:15	28	24	56	63	38	22	27	28	72	4,5
Day	14:15	54	22	49	24	23	33	36	22	66	4,1
May 19th 2005										69	4,3
D 0	15:55	7	5	9	9	6	6	13	6	15	1,0
	16:45	2	10	6	9	4	4	4	8	12	0,7
										14	0,8

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 20th 2005	09:40	11	16	15	16	11	13	12	22	29	1,8
D+1										29	1,8

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 21st 2005	13:30	76	50	69	22	60	40	67	74	115	7,2
D+2	15:00	36	45	44	36	41	36	56	25	80	5,0
										97	6,1

Day	Time	T1 a	T1 b	T2 a	T2 b	T3 a	T3 b	T4 a	T4 b	moy zT	T/m2
May 22nd 2005	13:00	109	82	110	16	53	31	50	37	110	6,8
D+3											

T : number of bees counted on the treated area
T/ m2 : number of mean bees per square meter
mean zT: number of mean bees per crop plot
D-3 to D-1: 3 to 1 days before application
DO: 0 days before application
D+1 to D+3: 1 to 3 days after application

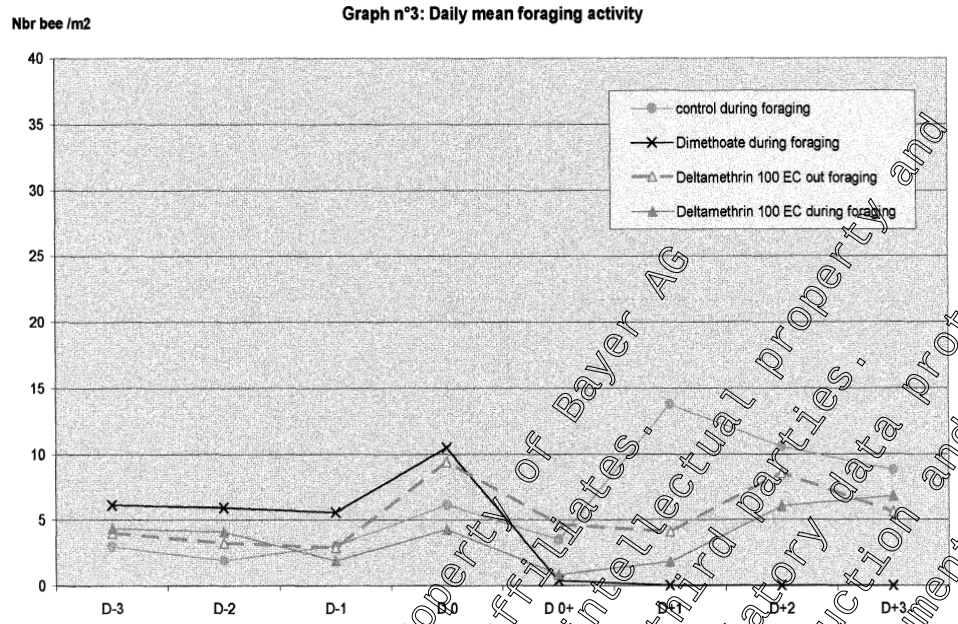


Figure 2: Daily mean foraging activity [Nbr bee/m²] for Deltamethrin 100 at 6.25 g a.s./ha during foraging, Deltamethrin 100 at 6.25 g a.s./ha out of foraging, control and the reference item (Dimethoate)

D-3 to D-1: 3 to 1 days before treatment
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+3: 1 to 3 days after treatment

Data were collected at least once a day and sometimes twice a day in order to confirm the trend of this foraging activity. On the day of application, 4 registered records of this activity were used to build up the graph, twice before application and twice after treatment.

According to this parameter, the quantity of forager bees was firstly similar among tunnels after the hive introduction. The bee activity level ranges from 3 to 6 bees per meter square at this time.

From 3DBT to D 0 the bee colonies reacted to the sugar spray solutions according to their needs and to the number of available forager honeybees. So the activity ranges to sometimes 10 bees per m^2 that was much over the required level. Yet, the sugar solution was daily sprayed in the morning and was less and less attractive along the day. The bees were very active soon after the spray when the staff came to count them. During 0DAT the data expressed the average of two counts before and after treatments.

The main information according to this foraging parameter was the decrease of the activity in the whole tunnels during the application day. The decrease in the control should was suggested as standardised because of the less attractiveness of the sugar solution. This decrease was similar in the tunnel where Deltamethrin 100 EC was applied out of the bee presence. On the contrary in the tunnels where Deltamethrin 100 EC and reference were applied the foraging activity drops to null in the afternoon.

During this second part of the trial the activity in the water control tunnel was regular and still over the others.

On the contrary, the reference dimethoate induced a high decrease in foraging activity soon after treatment. Moreover this impact runs on during the next three days and the level reaches the nullity. The repellence was so high that bees keep prostrated in their hives.

Colony Assessment

Few changes only appear on these parameters between the two visits. Because of the honey dew from the sugar solution, forager bees had an activity but the colonies present food storage at the end. By comparison the proportion of brood surface remained stable in the four hives.

According to the confinement under tunnels and to the short time between the two assessments these changes were non-significant in the tunnels.

Yet adult bee populations' decreased in all tunnels, only the control colony presents a small decrease in the bee population that allows a further development.

Conclusion:

Overall conditions for conducting this experimental phase of the study were favourable to the bee activity on the wheat plots. Climatic and crop conditions were satisfactory, so that observed parameters agree with the recorded data.

Bee colonies were strong enough and the design provides mortality data and foraging data and confirmed the reliability of the trial conditions in all tunnels.

Experimental conditions were quite strict, including confinement and spraying during foraging.

The water control and the reference dimethoate validated the results with standardised data. Mortality trend was regular in the control tunnel along the whole experimental period and the dimethoate induces a high peak of mortality that confirmed the toxicity of this reference.

In these experimental conditions, the study item Deltamethrin 100 EC (at the dose of 0.0625 L/ha) applied during the bee activity induced a discrete recorded effect on mortality on the following day after treatment only.

Compared to the reference dimethoate this impact on the mortality parameter was really limited. Raw data expressed several mortality levels and the calculation of the toxicity index suggested that this mortality level was comparable between Deltamethrin 100 EC and the water control.

When applied at night in absence of bees, Deltamethrin 100 EC (at the dose of 0.0625 L/ha) presented no impact on mortality at 1DAT and a stable foraging activity on the treated wheat plots when it was supposed to increase as in the control.

*****

Report:	**KCP 10.3.1.5/17 [redacted]; 2001**
Title:	Tunnel test - acute, short and medium term effects of AE F032640 00 EC11 A308, applied on cereals, on honey bees (*Apis mellifera* L.)
Document No.:	M-505203-01-1 (Rep. No.: S00AGB3264VO54)
Guidelines:	CEB 129
GLP:	yes

Material and methods:

~~Bees were confined within tunnels on winter wheat fields (cereals sprayed with sugar solution in order to provide food resources to the bees). After an acclimatization phase of five days, application was~~

performed during bee flight. The control was treated with water, the test item was applied at a rate of 0.0625 L/ha (corresponding to 6.25 g a.s./ha). As a non-toxic standard, Zolone Flo was used at a rate of 1.2 L/ha. There was one replicate per treatment group. The assessed endpoints were mortality, foraging and flight activity, storage of honey and pollen, behavior, and brood development.

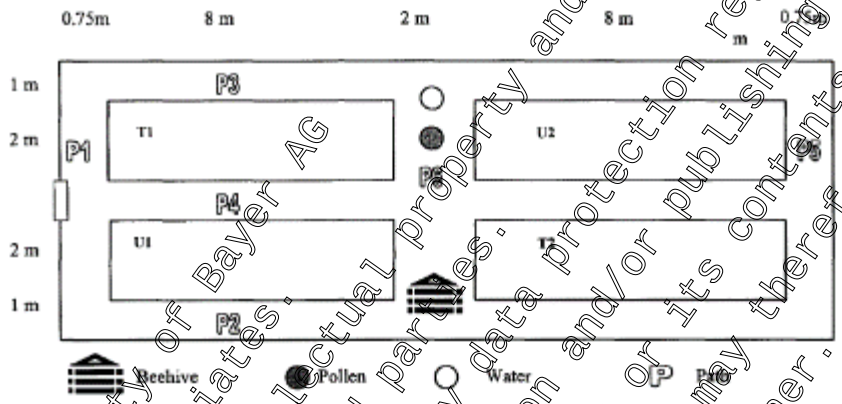
Findings:

Mortality was not affected by the test substance treatment. Foraging activity was likewise not or only very slightly affected by the test substance treatment on the treated as well as on the refuge areas in the tunnel. Furthermore, no effects on the behaviour were detected. Brood development was not affected by the test substance treatment as well.

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EC 100 (AE F032640 00 EC11 A308) content of a.s.: deltamethrin: 10.5 % w/w (100 g a.s./L nominal), density: 0.954 g/ml
Batch number:	08
Reference item:	Zolone Flo SC 500 (500 g a.s./L nominal, analysed content: 510 g a.s./L)
Test organism:	Honey bees (*Apis mellifera L*) The used hives were single box colonies (type DADANT 10 frames) with 10 frames, one queen and about 10000 bees per hive at test start. Queens were obtained by grafting (1 month) and colonies (consisting of Caucasian bees) were homogeneous as possible. Oldest worker honeybees were a maximum of 3 month old at test initiation. Additionally, an empty new frame of knowing weight was introduced in each hive prior their introduction into the tunnels. The corresponding queens hatched in 2000 and originated from one breeding line in order to guarantee uniform bee material in all treatment groups. Furthermore the following criteria for each colony were guaranteed: • 4 frames containing eggs, larvae and capped cells • 2 frames containing honey and pollen • 4 frames were kept empty for free space
Source:	[redacted] (Supplier)
Crop:	Winter wheat, cultivar Soissons sown October 21, 1999. Growth stage: BBCH 75-77
Test location:	[redacted] France [redacted] France. The apiary part was conducted [redacted]
Test unit:	Test was composed of 3 tunnels (control, test substance and non- toxic standard) Each tunnel covered an area of 136.5 m^2 (19.5 m × 7 m) with a height of approximately 3.5 m. The tunnel tent frames were covered with light plastic netting. Furthermore, each tunnel was divided into four areas (T1, T2 and U1, U2) of 16 m^2 (8 m × 2 m) each, containing the

crop. Areas T1 and T2 received treatment while U1 and U2 were refuge zones not receiving any treatment. The diagrammatic representation of a tunnel is shown in the following figure:



T1 and T2: treated areas
U1 and U2: refuge zones not receiving any treatment

Areas were separated from each other by a path for observation. In each tunnel one hive was placed and assignment to tunnels was randomised.

Application rates: Control (C): mineral water (Clarine).
Treatment rate (T): 0.0625 L/ha (6.25 g a.s./L) during foraging activity
Reference rate (R): 1.2 L/ha (600 g a.s./ha)

Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).

The spray volume was 300 L/ha in all treatment groups. The sprayer was calibrated before use. The deviation reached a maximum of 9.37%.

Data sampling: Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.

Data analysis: Not stated in the report.

Deviations from the study plan: No deviations to the study protocol.

Climatic conditions during the experiment

The environmental parameters recorded were within the normal range for the region. No dramatic weather conditions such as storms or violent winds occurred during study period. The environmental conditions are shown in table below.

Table 1: Field conditions

Date	Temperature Mini °C	Temperature Maxi °C	Relative air humidity % Mini	Maxi	Rainfall mm
10.05.00	14.6	23.7	54	97	3.8
11.05.00	14.3	22.2	62	98	0.8
12.05.00	12.8	23.5	48	98	-
13.05.00	11.1	23	51	98	-
14.05.00	12.5	26.7	45	99	-
15.05.00	14.7	27.8	42	95	-
16.05.00	11.6	25	56	95	-
17.05.00	8.9	17.5	45	92	-
18.05.00	7.7	17.4	48	94	2.2
19.05.00	3.4	15.5	53	95	-
20.05.00	5.1	18.2	37	99	-
21.05.00	4.8	17.2	48	96	1.2
22.05.00	7.8	18.8	49	98	-
23.05.00	8.5	21.5	49	99	-
24.05.00	11.7	21.8	44	91	0.2
25.05.00	7.1	18.5	43	96	-
26.05.00	7.2	20.9	49	98	-
27.05.00	7.9	18.3	35	91	3.6
28.05.00	4.2	17.9	38	96	-
29.05.00	7.2	17.7	40	98	1
30.05.00	11.6	16.4	81	98	14.6
31.05.00	10.8	19.2	61	98	-
01.06.00	13	22.4	63	97	-
02.06.00	14.5	29.2	54	98	-
03.06.00	17.2	25.4	69	98	0.4
04.06.00	12.9	21.5	61	97	12.6
05.06.00	9.7	14.7	82	97	3.6
06.06.00	8.4	18.5	66	98	-
07.06.00	9.4	21.1	41	98	-
08.06.00	11.2	26.9	37	90	-
09.06.00	12.6	24.9	49	97	12.8
10.06.00	9.6	18.4	55	96	-
11.06.00	8.1	21	48	97	-
12.06.00	10	25	53	98	-
13.06.00	15.3	27.7	46	98	-
14.06.00	14	25.7	53	97	-
15.06.00	14.3	27.6	51	98	-
16.06.00	12.7	25.4	57	93	-
17.06.00	11	26.5	37	97	-
18.06.00	13.2	31.1	32	87	-
19.06.00	13	32.1	37	96	-
20.06.00	15.8	30.7	38	95	-
21.06.00	13	24.8	52	96	-
22.06.00	12.7	23.2	38	93	-
23.06.00	13.2	21.2	46	90	-
24.06.00	11	18.3	46	94	-
25.06.00	9.9	24.9	34	90	-
26.06.00	9.4	22.6	43	90	-
27.06.00	11.1	23.5	31	83	0.4
28.06.00	13	25.3	31	82	-
29.06.00	12.6	26.2	35	86	-
30.06.00	14.4	28.5	34	89	-

Pesticide history of the field site:

Previous pesticide history of the test site is listed in the table below.

Table 2: Maintanance and pesticide history of the field site

Year	Crop	Pesticides			
		Herbicides Name (a.i.), rate	**Fungicides** Name (a.i.), rate	**Insecticides** Name (a.i.), rate	**Other**
2000	Winter wheat	**Celio** (March 7) (clodinafop-propargyl+ cloquincet-methyl) 0.6 L/ha **Agral 90** (March 7) 1 L/ha	**Unix** (April 18) (cyprodinil) 1 kg/ha **Amistar** (May 9) (azoxystrobine) 0.8 L/ha **Ogam** (May 26) (kresoxim-methyl+epoxiconazol) 0.8 L/ha	**Karate vert** (May 9) (lambda-cyhalothrin) 0.125 L/ha	
1999	Winter wheat	**Starane** (fluroxypyr) 0.5 L/ha **Chardax** (clopyralid+2,4-MCPA) 1.5 L/ha	**Ogam** (kresoxim-methyl+epoxiconazol) 0.6 L/ha **Caramba** (metconazole) 1 L/ha	**Gaucho blé** (imidacloprid+ bitertanol+anthraquinone) 0.4 L/100 kg seeds **Karate vert** (lambda-cyhalothrin) 0.125 L/ha	
1998	Corn	**Gesaprime** (atrazine) 3 L/ha **Mikado** (sulcotrione) 0.8 L/ha		**Gaucho** (imidacloprid) 0.07kg/150000 seeds **Karate** (lambda-cyhalothrin) 0.125 L/ha	-
1997	Winter wheat	**Starane** (fluroxypyr) 0.5 L/ha **Chardax** (clopyralid+2,4-MCPA) 1.5 L/ha	**Alto** (cyproconazol) 0.8 L/ha **Alto Marathon** (cyproconazole+chlorothalonil) 2 L/ha	**Gaucho blé** (imidacloprid+ bitertanol+ anthraquinone) 0.4 L/100 kg seeds **Karate vert** (lambda-cyhalothrin) 0.125 L/ha	-

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EC 100 on the honeybee, *Apis mellifera* under forced exposure conditions.

This study included three exposure groups (tunnels) each: one tap-water treated control group (C), one test-item group (T) and one reference item group (R). In all exposure groups, the crop was sprayed 5 days after set-up of the hives in the tunnels (Acclimatisation phase) at BBCH 75 - 77 (full-flowering), during honeybees actively foraging on the crop under confined conditions. The honeybees remained 13 days in the tunnels.

Depending on the weather conditions the observations were conducted for a 9 day period following a 4-day adaptation period of the hives to the confinement. At the end of this 9 day period, symptoms of toxicity (mortality, behaviour, etc.) were not observed in the Deltamethrin EC 100 treatment, the exposure phase of the study was stopped and beehives returned to the apiary.

The assessments of the number of any dead bees were performed in each tunnel, each morning during both the adaptation period of the hives and thereafter until the end of the test. For each tunnel, these daily assessments was performed commencing June 19 at approximately 36 hours after the introduction of the hive and subsequent daily assessment was conducted at approximately the same time for each tunnel each day. During each assessment all dead bees were collected in the 6 paths and in the dead bee trap (the bees collected from each of the path areas 1 to 5 were pooled).

The assessments of the foraging activity were performed only on those days when the weather was such that honeybees are likely to be foraging. Counting was done over the whole of the zone by assessing the number of bees passing in an area of 60cm wide, whilst walking at a normal speed down the side of the zone. The foraging activity of the bees was expressed as the number of bees observed separately within each zone on each assessment. After the adaptation period of the beehive, the foraging activity was evaluated twice a day at regular intervals (starting around 10 a.m. in the morning and 3 p.m. in the afternoon). In addition, the day of treatment foraging activity is assessed around 15 minutes before treatment and around 15 minutes and 1 hour after each treatment.

Behaviour of bees was observed during assessment of bee mortality, foraging activity and control of the colony. Bees were observed for abnormalities like aggressiveness, intensive flying without landing on the crop, moribund or severely affected bees, bee accumulation at the beehive entrance, trembling bees, no longer producing pollen balls, etc..

Assessments on the condition of the colony were made on the day of their installation within the tunnels, June 17, on the middle of the exposure phase, June 26, just after returning bee hives to the beekeeper, June 30, and around one month after the return to the apiary, August 8.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Cumulative number of dead bees before as well as after the applications in the control, the test item group and the reference item group, respectively
- Number of foraging bees per zone (T1, T2 and U1, U2) and number of bee/m² in each tunnel before as well as after the applications in the control, the test item group and the reference item group, respectively.
- Behaviour of the bees during assessments of bee mortality, foraging activity and the control of the colony. In addition the date time and duration of such abnormal behaviours was recorded.
- Control of the colony with the following criteria examined: weight of the empty frame introduced into the centre of the hive, for both sides of each frame the percentage frame surface area containing honey, for both sides of each frame the percentage frame surface area containing pollen, for both sides of each frame the percentage frame surface area containing eggs, for both sides of each frame the percentage surface area of brood (young and old larvae) in each frame and % of capped and uncapped alveolus as well as the health of the queen.

Dates of Work: 17th June to 08th August 2000

Findings:

Honey bee mortality:

A summary of the daily mortality (cumulated dead bees) is shown in following table.

Table 3: Cumulated dead bees during the study period (only males and worker-bees considered)

Date	Tunnel No. 1 Deltamethrin EC 100 @ 6.25 g a.s./ha			Tunnel No. 2 Water			Tunnel No. 4 Zolone Flo @ 600 g a.s./ha		
	Males	Workers	Total	Males	Workers	Total	Males	Workers	Total
19.06.00 3DBT	24	891	**915**	12	244	**256**	14	890	**904**
20.06.00 2DBT	27	994	**1021**	27	423	**450**	23	1162	**1185**
21.06.00 1DBT	30	1077	**1107**	30	475	**505**	56	1294	**1350**
22.06.00 0DBT	30	1114	**1144**	33	494	**527**	66	1400	**1466**
22.06.00 0DAT	30	1152	**1182**	33	513	**546**	73	1466	**1539**
23.06.00 1DAT	30	1168	**1198**	34	524	**558**	79	1478	**1557**
24.06.00 2DAT	31	1219	**1250**	35	551	**586**	86	1788	**1874**
26.06.00 4DAT	37	1360	**1397**	41	692	**733**	119	2099	**2218**
27.06.00 5DAT	39	1480	**1519**	42	812	**854**	138	2425	**2563**
28.06.00 6DAT	40	1606	**1646**	43	938	**981**	140	2622	**2762**
29.06.00 7DAT	40	1678	**1718**	44	957	**1001**	141	2905	**3046**
30.06.00 8DAT	40	1740	**1780**	44	999	**1043**	141	3143	**3284**

DBT: days before treatment
DAT: days after treatment

The effect of Deltamethrin EC 100 on bee mortality was nil and even lower than the bee mortality of the non-toxic standard Zolone Flo. When beehives returned to the apiary no mortality was observed.

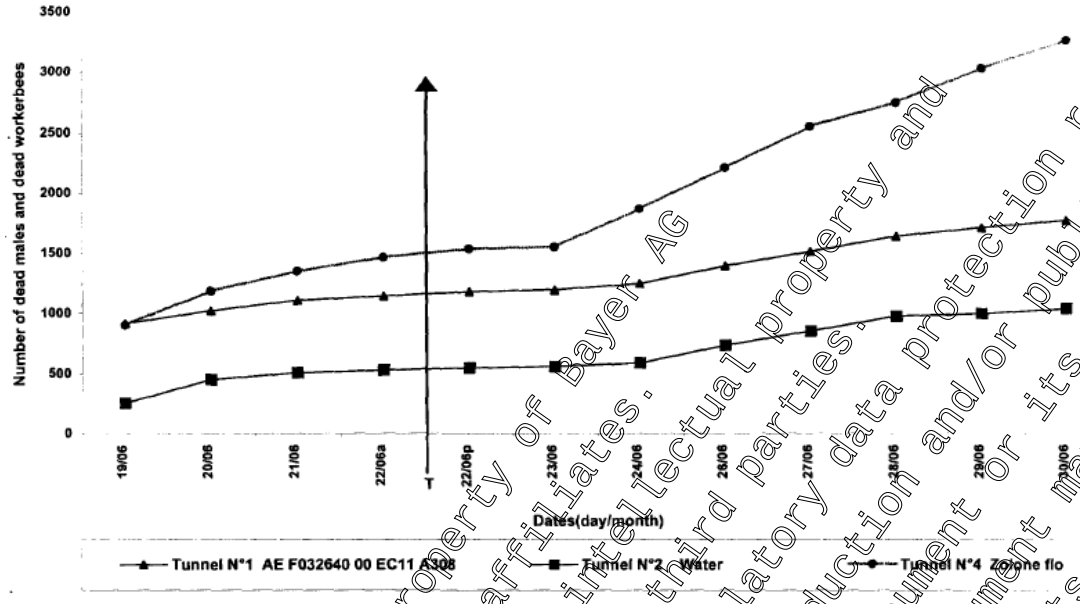


Figure 1: Cumulative mortality of bees (T= Treatment) in the different tunnels: Deltamethrin EC 100 (AE F032640 00 EC11 A308), water control and Zolone flo

Honey bee flight intensity:

Foraging activities, in cereals, are basically low in all tunnels. Deltamethrin EC 100 had no or very limited effect on the foraging activity in the days following the treatment on both treated and refuges areas.

Comparisons between the different treatments and treated zones and refuge zones are presented in the following tables.

Table 4: Number of bees foraging in the treated zones (T1, T2) in the different tunnels: Deltamethrin EC 100 (AE F032640 00 EC11 A308), water control and Zolone flo

Assessment date (Day/month/hour)	Number of bees/m² (means)		
	Tunnel N°1 AE F032640 00 EC11 A308	Tunnel N°2 Water	Tunnel N°4 Zolone flo
21/06/10h11-10h35	1.53	1.41	1.50
21/06/15h05- 15h31	1.13	1.06	2.91
22/06/9h30-10h08	3.03	2.41	5.66
22/06/11h04-12h15	2.5	1.88	1.44
	Treatments		
22/06/11h44-12h47	1.03	0.97	0.38
22/06/12h44-13h35	0.19	0.88	0.38
22/06/16h03-16h28	0.13	0.19	0.30
23/06/10h15-10h40	1.78	2.31	1.94
23/06/15h08-15h31	0.75	2.53	1.09
24/06/10h05-10h59	1.69	1.25	1.63
26/06/10h08-10h34	1.44	1.78	1.44
26/06/15h51-16h15	1.78	1.31	1.91
27/06/10h04-10h28	1.34	2.97	1.94
27/06/15h09-15h36	3.56	3.56	3.63
28/06/10h11-10h49	6.69	6.84	8.09
28/06/15h59-16h08	2.13	2.88	1.28
29/06/10h59-11h26	2.16	2.00	0.97
29/06/15h50-16h12	1.09	1.34	1.31
30/06/10h27-10h56	4.34	7.47	3.13
30/06/15h15-15h37	0.91	0.59	0.75

Table 5: Number of bees foraging in the refuge zones (U1, U2) in the different tunnels: Deltamethrin EC 100 (AE F032640 00 EC11 A308), water control and Zolone flo

Assessment date (Day/month/hour)	Number of bees/m² (means)		
	Tunnel N°1 AE F032640 00 EC11 A308	Tunnel N°2 Water	Tunnel N°4 Zolone flo
21/06/10h11-10h35	3.34	2.03	1.66
21/06/15h05- 15h31	0.88	0.94	3.47
22/06/9h30-10h08	2.34	2.13	5.22
22/06/11h04-12h15	2.00	1.69	1.16
	Treatments		
22/06/11h44-12h47	1.34	1.06	1.34
22/06/12h44-13h35	0.22	0.56	0.50
22/06/16h03-16h28	0.22	0.28	0.75
23/06/10h15-10h40	2.13	1.94	3.13
23/06/15h08-15h31	1.34	2.56	1.47
24/06/10h05-10h59	2.19	1.25	1.75
26/06/10h08-10h34	1.03	1.56	1.28
26/06/15h51-16h15	1.63	1.25	1.72
27/06/10h04-10h28	1.82	3.00	2.19
27/06/15h09-15h36	3.25	2.97	4.13
28/06/10h11-10h49	5.75	6.59	6.44
28/06/15h59-16h08	1.78	1.81	1.63
29/06/10h59-11h26	1.00	1.47	0.78
29/06/15h50-16h12	0.69	1.59	0.69
30/06/10h27-10h56	2.69	6.03	3.28
30/06/15h15-15h37	0.75	0.72	1.44

Behaviour of the bees

Deltamethrin EC 100 had no effect on the bee behaviour in the days following the treatment and in the month after beehives returned to the apiary.

Control of the colony

Reserves and brood were reduced during the study in all the tunnels, which is typical of such a study. The test item Deltamethrin EC 100 had no negative effect on the control of the colony and this at each date of assessment.

Assessments of the control of the colony are listed in the following tables.

Table 6: Control of the colony exposed to Deltamethrin EC 100 treated wheat

Observations		Frame N°1 N° VO54-2-C1				Frame N°2 N° VO54-2-C2				Frame N°3 N° VO54-2-C3				Frame N°4 N° VO54-2-C4				Frame N°5 N° VO54-2-C5				Frame N°6 N° VO54-2-C6				Empty frame			
Date		17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	350	-	350	300
% frame surface area containing honey	Side a	10	20	0	10	10	10	30	60	5	10	5-10	20	5	10	5-10	20	10	10	30	30	20	10	15	30	0	0	0	0
	Side b	15	20	30	0	10	20	30	40	0	5	5-10	20	1	5	5-10	30	30	20	10	30	0	0	0	20	0	0	0	0
% frame surface area containing pollen	Side a	0	0	0	0	0	0	0	30	5	5	0	0	0	0	0	15	30	10	2	15	0	0	0	10	0	0	0	0
	Side b	0	0	0	0	0	0	0	5	5	5	0	0	0	0	0	30	30	0	0	0	0	0	0	5	0	0	0	0
% frame surface area containing eggs	Side a	NR	NR	NR	NR	NR	NR	NR	NR	5	5	0	NR	15	15-20	0	NR	20	NR	10	NR	NR	NR	0	NR	NR	NR	NR	NR
	Side b	NR	NR	NR	NR	NR	NR	NR	NR	10	10	0	NR	15	20	0	50	0	NR	0	NR	NR	NR	0	NR	NR	NR	NR	NR
% surface area of brood	Side a	60	0	0	0	60	60	30	0	60	30	30	70	70	70	30	50	60	20	5-10	40	0	0	0	60	0	0	0	0
	Side b	0	0	0	0	60	50	10	0	70	10	10	60	60	60	30	60	40	30	10	60	0	0	0	70	0	0	0	0
% capped alveolus	Side a	100	NR	NR	NR	20	20	100	NR	60	10	10	30	90	20	10	70	50	50	100	90	NR	NR	NR	90	NR	NR	NR	NR
	Side b	NR	NR	NR	NR	20	20	100	NR	80	20	30	70	80	40	0	80	0	0	100	90	NR	NR	NR	80	NR	NR	NR	NR
% uncapped alveolus	Side a	NR	NR	NR	NR	80	80	0	NR	40	80	30	20	10	60	80	30	50	50	0	10	NR	NR	NR	10	NR	NR	NR	NR
	Side b	NR	NR	NR	NR	80	50	0	NR	20	70	80	30	20	60	50	20	100	100	0	10	NR	NR	NR	20	NR	NR	NR	NR
		T				T				T				T				T				T				T			

NR: Not relevant; T: Treatment

Table 7: Control of the colony exposed to the water treated wheat

Observations		Frame N°1 N° VO54-1-C1				Frame N°2 N° VO54-1-C2				Frame N°3 N° VO54-1-C3				Frame N°4 N° VO54-1-C4				Frame N°5 N° VO54-1-C5				Frame N°6 N° VO54-1-C6				Empty frame			
Date		17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8	17.6	26.6	30.6	8.8
Weight in g		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	750	-	750	2000
% frame surface area containing honey	Side a	10	10	0	60	10	10	30	30	15	15	0	30	5-10	10	15	30	15	15	30	30	15	15	5-10	30	0	0	0	70
	Side b	0	10	50	30	10	10	50	30	10	15	0	30	10	10	10-15	20	10	20	10	30	15	15	5-10	30	0	0	0	80
% frame surface area containing pollen	Side a	0	0	0	10	10	10	0	5	30	5	0	10	0	0	0	5	0	0	0	15	0	10	80	15	0	0	0	20
	Side b	10	0	0	5	20	20	0	20	0	0	0	5	0	5	0	5	0	0	0	0	0	15	80	0	0	0	0	0
% frame surface area containing eggs	Side a	NR	NR	NR	NR	5	2-5	NR	NR	5	5	10	50	5-10	0	10	0	15	0	NR	0	NR	NR	NR	0	NR	NR	NR	NR
	Side b	NR	NR	NR	0	NR	0	NR	NR	0	10	50	NR	0	0	0	NR	0	NR	0	NR	NR	NR	10	NR	NR	NR	NR	NR
% surface area of brood	Side a	0	5	0	0	30	30	10	70-60	50	50	80	50	50	50	80	70	15	15	30	60	5-10	5	0	60	0	0	0	0
	Side b	0	0	0	50	30	20	15	60	60	60	80	50	60	60	80	50-60	0	10	20-30	50-60	2	0	0	70	0	0	0	0
% capped alveolus	Side a	NR	NR	NR	NR	50	50	100	100	80	80	0	0	80	80	80	70	0	80	100	90	60	100	NR	70	NR	NR	NR	NR
	Side b	NR	NR	NR	20	50	60	100	100	60	60	0	0	50	50	80	10-15	NR	80	100	90	50	NR	NR	10	NR	NR	NR	NR
% uncapped alveolus	Side a	NR	NR	NR	NR	45	45	0	0	20	20	100	100	10	10	20	30	100	10	0	5	40	0	NR	30	NR	NR	NR	NR
	Side b	NR	NR	NR	80	40	40	0	0	40	40	100	100	50	50	20	80	NR	20	0	10	50	NR	NR	90	NR	NR	NR	NR
		T				T				T				T				T				T				T			

NR: Not relevant; T: Treatment

Conclusion:

The effect of Deltamethrin EC 100 on bee mortality was nil and even lower than the bee mortality of the non-toxic standard Zolone Flo for which a slight increase in mortality was observed two to three days after application. When beehives returned to the apiary no mortality was observed.

Deltamethrin EC 100 had no or limited effect on the foraging activity in the days following the treatment on both treated and refuges areas.

Furthermore, Deltamethrin EC 100 had no effect on the bee behaviour in the days following the treatment and in the month after beehives returned to the apiary.

Reserves and brood were reduced during the study in all the tunnels, which is typical of such a study. Deltamethrin EC 100 had no negative effect on the control of the colony and this at each date of assessment.

*****

Report:	**KCP 10.3.1.5/18, [redacted]; 2000**
Title:	Impact of AE F032640 00 EW01 B106 on bumblebees (*Bombus terrestris*) (insectproof tunnels on *Phacelia* crop) Code: AE F032640 00 EW01 B106
Document No:	M-200040-01-1 (Rep. No.: 2000-24.1)
Guidelines:	CEB 129
GLP:	yes

Material and Methods:

~~Bumblebee colonies were confined in tunnels on *Phacelia* fields, with two hives per tunnel. The test was replicated once. Six days after introduction, application of the test substance was performed (at 0.833 L/ha) during bumble bee flight, as well as application of the control, and of a phosalone standard. The assessed Endpoints assessed were foraging activity, mortality, and behavior.~~

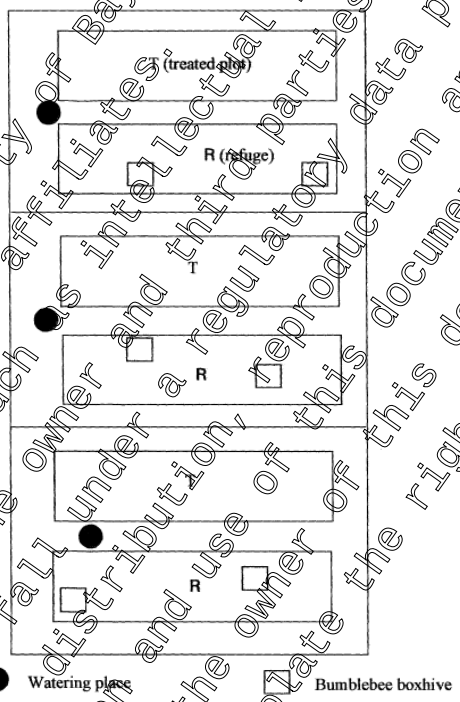
~~Findings:~~

~~Foraging activity of the bumble bees was only slightly affected by the treatment of test substance and only for a short time. Mortality and behaviour were not affected at all.~~

Material and Methods:

Test material:	Deltamethrin
Test item:	Deltamethrin EW 15 G (AE F032640 00 EG06 A107), content of a.s.: deltamethrin: 1.51 % w/w (15.0 g a.s./L nominal), density: 1.023 g/ml
Batch number:	TA151/99PM
Reference item:	Zolone Flo (active ingredient: phosalone, 500 g a.s./L nominal, analysed content: 499 g/L)
Test organism:	Bumblebees (*Bombus terrestris*) Populations were estimated at around 60 to 80 bumblebees per hive.
Source:	Bumblebee colonies came from a specialised society, breeding bumblebees for pollination.
Crop:	*Phacelia tanacetifolia* variety: TITAN (bee attractive crop) at flowering stage.
Test location:	[redacted] on a field [redacted] France
Test unit:	Each tunnel had a half-moon support made from galvanised steel; the hoops were nailed in the soil and joined with crossbars.

The surface was divided in 3 parts with partition sheets. Each unit was 42 m^2 (7 m x 6 m) and their roof height approximately 3 metres. A polyethylene mesh net (1.2 mm x 1.2 mm) covered the supports. Access was possible through a zip opening.
Inside the tunnels, the phacelia crop in each unit was split into 2 plots. Each had a surface of 7.5 m^2 (5 m x 1.5 m), first plot was considered as sheltered areas (R1 and R2; not treated with test item), the other two (T1 and T2) as treated areas.
Two bumblebee hives were placed in each unit where a watering place was supplied daily.
Exact interior design of the tunnel is shown in the figure below:



R: refuge (sheltered) area
T: treated area

Application rates: Control (C): Tap water
Treatment rate: 0.833 L/ha (12.5 g a.s./ha) during foraging activity
Reference rate (R): 1.2 L/ha (600 g a.s./ha)
Every treatment comprised of one replicate (i.e. 1 tunnel per treatment).
The spray volume was 300-315 L/ha in all treatment groups. The sprayer was calibrated before use.

Data sampling: Data for mortality, foraging activity, behaviour of the bees and data of the colony were assessed.

Data analysis: All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.

Deviations from the study plan: No deviation mentioned.

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperatures favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table.

Table 19: Weather data

	Temperature mini (°C)	Temperature maxi (°C)	Rainfall (mm)
August 22nd 2000	7	25	0
August 23rd 2000	14	30	0
August 24th 2000	14	33	0
August 25th 2000	17	35	0
August 26th 2000	14	25	0
August 27th 2000	15	25	12
August 28th 2000	11	24	0
August 29th 2000	9	25	0
August 30th 2000	14	20	0
August 31st 2000	12	26	4.5
September 1st 2000	14	23	5
September 2nd 2000	14	23	0
September 3rd 2000	12	21	0
September 4th 2000	6	20	0

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 2: *Phacelia* crop data

Date	Operation	Characteristics
May	Soil preparation	Herbicide application and harrowing : seedbed preparation and weed destruction
15/05/00	plot sawing + rolling	Specie *Phacelia tanacetifolia* variety : TITAN Reference : FG 53 / 777 06 – 1998 OECD system, EC norms, CTA method Model : 580_427-584 Dose seed 6,0 kg per ha
22/09/99	Destruction	Crushing the crop on experimental plots

The effects of Deltamethrin EW 15 G were tested on the bumblebees (*Bombus terrestris*) under confined semi-field conditions by following the guidance document C.E.B. method no. 129.

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EW 15 G on the bumblebees (*Bombus terrestris*) under forced exposure conditions.

This study included three exposure groups (tunnels) with one replicate (tunnel) each: one water treated control group, one test-item group and one reference item group. Two bumblebee boxhives were introduced into each elementary unit 6 days before product applications in order to enable the colonies

to adapt to their environment. Bumblebee colonies were submitted to test substances while foraging on sprayed crops. Experimental conditions of this type of study were very strict because the colonies are confined in tunnel parts.

Mortality in each tunnel unit was recorded on a daily basis for all areas covered with plastic film, from days 5DBT to 7DAT. Moreover, the day on which product application was carried out (day 0) additional counts were done at the end of the day (0DAT) in order to establish possible brutal intoxication of foraging bumblebees. The total mortality rate recorded in a tunnel unit for a given day results from adding up mortality rates observed in each of the plastic rows in the unit.

Foraging was observed from 2DBT to 3DAT, on all treated and sheltered areas. It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts could be shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall...etc.) This parameter was also taken into account for an additional count on the day of treatment, during the hour following product application.

Observations on behaviour were carried out during the trial in order to better understand the incidence of pesticide application on bumblebee behaviour. But these observations appeared especially important on the day the products were applied. On this time and during the thirty minutes following product application, bumblebee reactions and behaviour in each of the tunnels were observed (intense flying, clusters on the net or at the entrance of the box hive, aggressiveness, beginning of an intoxication...). In general, this observation phase continued all over the day, between counts, and results were compared to usual activities before product application.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item group and the reference item group, respectively
- Number of foraging bees/m² per day on all the areas (T and R) before as well as after the applications in the control, the test item group and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the test item groups and the reference item group, respectively

Dates of Work: August 22nd to September 4th 2000

Findings:

Mortality:

A summary of the daily mortality and total mortality results are shown in the following tables.

Table 3: Daily mortality data

Total	Deltamethrin EW 15 at 12.5 g a.s./ha	Zolone Flo	Water control
5DBT -23th August	1	0	0
4DBT- 24th August	1	1	0
3DBT- 25th August	0	2	0
2DBT- 26th August	1	0	0
1DBT - 27th August	0	0	2
0DBT - 28th August	1	0	0
0DAT - 28th August	0	0	0
1DAT - 29th August	2	0	0
2DAT - 30th August	1	0	1
3DAT – 31st August	1	0	0
4DAT – 1st September	1	0	1
5DAT – 2nd September	1	0	1
6DAT – 3rd September	1	0	0
7DAT – 4th September	0	1	0

DBT: days before treatment
DAT: days after treatment

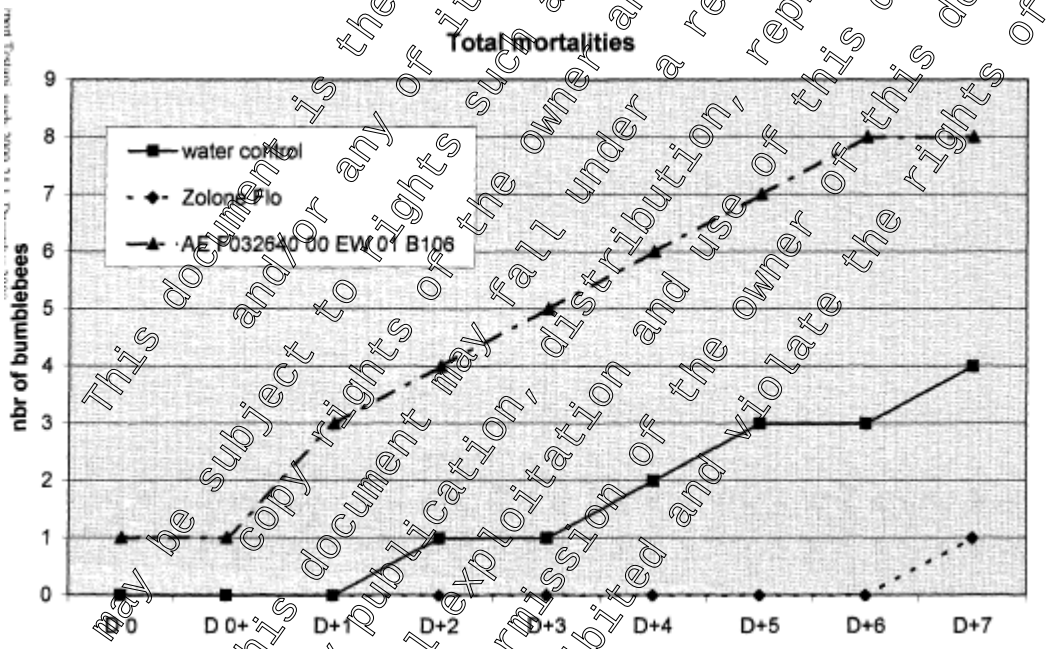


Figure 1: Total mortalities for the reference group (Zolone Flo), Deltamethrin EW 15 (AEF032640 00 EW01 B106) at 12.5 g a.s./ha and for the water control group
D0: 0 days before treatment
D0+: 0 days after treatment
D+1 to D+7: 1 to 7 days after treatment

Daily mortality did not increase in any modality after treatment. Only one or two individuals were collected a day in Deltamethrin EW 15 G modality. However the difference occured while there was absolutely no mortality in the reference modality. In the control tunnel (treated with water) the colony was no more disturbed by the treatment. Mortality rates recorded varied very few along the week.

Only total mortalities seemed dissimilar after seven days post treatment as the graph shows. In this graph curves were therefore all increasing, records were taken into account from days of application (day 0) in order to understand the impact of product applications.

Foraging:

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following tables.

Table 4: Foraging data: Deltamethrin EW 15 at 12.5 g a.s./ha

AEF032640 EW01 B106		number of bumble-bees per zone		calculated data	
day	time	R	T	R / m²	T / m²
26 août 00	13h00	22	29	2,9	3,9
D-2	15h00	19	26	2,5	3,5
	16h30	17	25	2,3	3,3
					3,6
27 août 00	10h00	14	38	1,9	5,1
D-1	11h00	44	41	5,9	5,5
	12h00	47	49	6,3	6,5
					5,7
28 août 00	11h00	44	46	5,9	6,1
D 0	11h30	46	52	6,1	6,9
					6,5
	13h00	29	26	3,9	3,5
	15h30	44	49	5,9	6,5
	16h45	62	52	8,3	6,9
					5,6
29 août 00	10h00	50	51	6,7	6,8
D+1	11h00	57	54	7,6	7,2
	12h00	55	61	7,3	8,1
					7,4
30 août 00	10h00	48	52	6,4	6,9
D+2				0,0	0,0
				0,0	0,0
					6,9
31 août 00	10h00	35	32	4,7	4,3
D+3	11h00	57	39	7,6	5,2
	12h00	64	62	8,5	8,3
					5,9

R : number of bumblebees counted on sheltered area by an experimentator
T : number of bumblebees counted on the treated area by an experimentator.
R / m² : number of mean bumblebees per metre square on R (sheltered area).
Precision calculation : R / m² = R / 7.5
Precision calculation : T / m² = T/ 7.5
D-2/ D-1: 2/ 1 days before application
DO: 0 days before application
D+1/ D+2/ D+3: 1/ 2/ 3days after application

Table 5: Foraging data: Zolone Flo

Zolone Flo		number of bumble-bees per zone		calculated data	
day	time	R	T	R / m²	T / m²
26 août 00	13h00	15	9	2,0	1,2
D-2	15h00	17	10	2,3	1,3
	16h30	14	9	1,9	1,2
					1,2
27 août 00	10h00	20	21	2,7	2,8
D-1	11h00	26	19	3,5	2,5
	12h00	24	20	3,2	2,7
					2,7
28 août 00	11h00	18	21	2,4	2,8
D 0	11h30	29	22	3,9	2,9
					2,9
	13h00	32	17	4,3	2,3
	15h30	33	27	4,4	3,6
	16h45	33	26	4,4	3,5
					3,5
29 août 00	10h00	19	16	2,5	2,1
D+1	11h00	19	21	2,5	2,8
	12h00	15	24	2,0	3,2
					3,2
30 août 00	10h00	36	23	4,8	3,1
D+2				0,0	0,0
				0,0	0,0
					3,1
31 août 00	10h00	18	19	2,4	2,5
D+3	11h00	20	26	2,7	3,5
	12h00	26	30	3,5	4,0
					3,3

R : number of bumblebees counted on sheltered area by an experimentator
T : number of bumblebees counted on the treated area by an experimentator
R / m^2 : number of mean bumblebees per metre square on R (sheltered area).
Precision calculation : R / m^2 = R / 7,5
Precision calculation : T / m^2 = T/ 7,5
D-2/ D-1: 2/ 1 days before application
DO: 0 days before application
D+1/ D+2/ D+3: 1/ 2/ 3days after application

Table 6: Foraging data: Water Control

Water control		number of bumble-bees per zone		calculated data	
day	time	R	T	R / m²	T / m²
26 août 00	13h00	22	17	2,9	2,3
D-2	15h00	19	13	2,5	1,7
	16h30	14	13	1,9	1,7
					1,9
27 août 00	10h00	36	26	4,8	3,5
D-1	11h00	32	28	4,3	3,7
	12h00	23	21	3,1	2,8
					3,7
28 août 00	11h00	34	27	4,5	3,6
D 0	11h30	24	28	3,2	3,7
					3,7
	13h00	36	36	4,8	4,8
	15h30	35	37	4,7	4,9
	16h45	30	44	4,0	5,9
					5,2
29 août 00	10h00	30	26	4,0	3,5
D+1	11h00	45	37	6,0	4,9
	12h00	49	43	6,5	5,7
					4,7
30 août 00	10h00	41	34	5,5	4,5
D+2				0,0	0,0
				0,0	0,0
					4,5
31 août 00	10h00	40	39	5,3	5,2
D+3	11h00	47	26	6,3	3,5
	12h00	53	35	7,1	4,7
					4,4

R : number of bumblebees counted on sheltered area by an experimentator
T : number of bumblebees counted on the treated area by an experimentator.
R / m^2 : number of mean bumblebees per metre square on R (sheltered area)
Precision calculation : R / m^2 = R/ 7.5
Precision calculation : T / m^2 = T/ 7.5
D-2/ D-1: 2/ 1 days before application
DO: 0 days before application
D+1/ D+2/ D+3: 1/ 2/ 3days after application

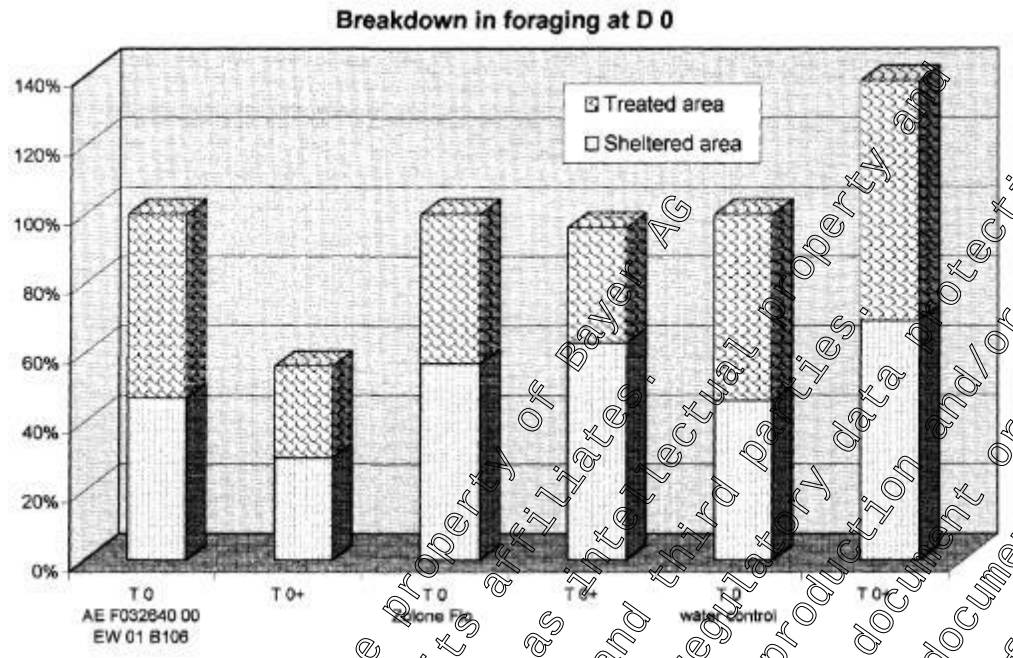


Figure 2: Breakdown of foraging on treatment day for the reference group (Zolone Flo). Deltamethrin EW 15 (AEF032640 00 EW01 B106) at 12.5 g a.s./ha and for the water control group
T0: before treatment
T0+: after treatment

In the morning of the product application day, foraging was already quite active in the 3 modalities and quite similar one another. The level of this foraging activity was again 3 to 6 bumblebees per m^2. During the three counts that followed product application, mean foraging trends were a bit different between modalities. In fact, foraging activity remained stable in the reference modality where spraying did not disturb the foragers' activity. However, in the unit where Deltamethrin EW 15 formulation was applied the activity decreases a few but didn't stop and the average level in the afternoon was therefore over 5 bumblebees per metre square. The bumblebee colony in the water control modality seemed indifferent to water application and foraging increased during the day over pre-treatment phase level.

Colony behaviour:

In such a test, with homogeneous bumblebee colonies, behaviour was also comparable between modalities, as foraging was quite regular on phacelia plots. Bumblebee foragers only showed little reaction to treatments in the different modalities. The volume of a unit modality represented sufficient flight space but it was nevertheless confined and colonies adapt to this environment after the first recordings.

From the beginning of this experimental phase, plots were very attractive for foragers and this triggers activity of bumblebees within box hives. During spraying, the bumblebees present on the experimental plot when the boom passes flight away over treated plot. Generally they come back again a little further away. Experimentators did not notice neither any particular aggressiveness nor any frenetic bumbling.

Conclusion:

Overall conditions for conducting this experimental phase of the scheme were favourable to bumblebee activity. Climatic and crop conditions were satisfactory. The different parameters observed agree with the results obtained.

Experimental conditions of the study were quite strict, including confinement and product application carried out during intense foraging activity, on attractive plots.

The use of any phyto-pharmaceutical substance did not give any high mortality stage.

The effects of the test substance Deltamethrin EW 15 in the case of this trial on a phacelia crop, only showed a temporary decrease in foraging, and no impact on daily mortality.

*****

Report:	**KCP 10.3.1.5/19, [redacted]; 2000**
Title:	Impact on bumblebees (*Bombus terrestris*) (insectproof tunnels on *phacelia* crop) Code: AE F032640 00 EG0G06 A107
Document No:	M-200043-01-1 (Rep. No.: 2000-24.3)
Guidelines:	CEB 129
GLP:	yes

~~**Material and Methods:**~~

~~Bumblebee colonies were confined in tunnels on *Phacelia* fields, with two hives per tunnel. The test was replicated once. Six days after introduction, application of the test substance was performed (at 0.2 kg/ha) during bumble bee flight, as well as application of the control and of a phosalone standard. The assessed endpoints were foraging activity, mortality and behavior.~~

~~**Findings:**~~

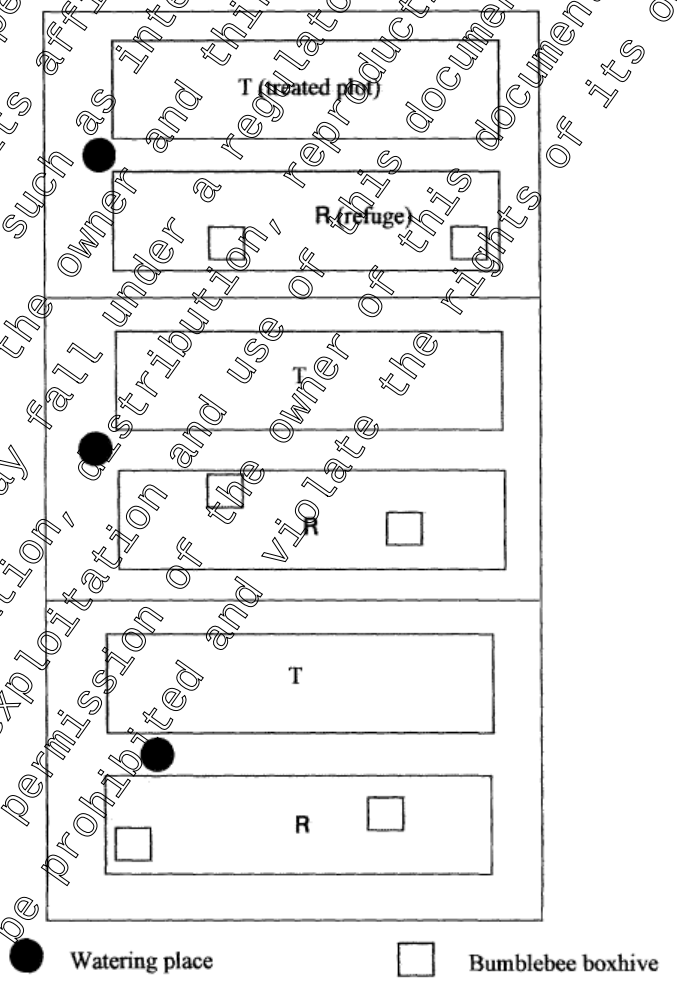
~~Foraging activity of the bumblebees was only slightly affected by the treatment of test substance and only for a short time. Mortality and behavior were not affected at all.~~

Material and Methods:

Test material	Deltamethrin
Test item:	Deltamethrin EG 6.25 W (AE F032640 00 EG06 A107), 6.14 % w/w (62.5 g a.s./kg nominal).
Batch number:	8FES0248
Reference item:	Zolone Flo (active ingredient: phosalone, 500 g a.s./L nominal, analysed content: 499 g a.s./L)
Test organism:	Bumblebees (*Bombus terrestris*) Populations were estimated at around 60 to 80 bumblebees per hive.
Source:	Bumblebee colonies came from a specialised society, breeding bumblebees for pollination.
Crop:	*Phacelia tanacetifolia* (bee attractive crop) of the TITAN variety at flowering stage.

Test location: [redacted] on a field [redacted] France

Test unit: Each tunnel had a half-moon supports made from galvanised steel; the hoops were nailed in the soil and joined with crossbars. The surface was divided in 3 parts with partition sheets. Each unit was 42 m^2 (7 m x 6 m) and their roof height approximately 3 metres. A polyethylene mesh net (1.2 mm x 1.2 mm) covered the supports. Access was possible through a zip opening.
Inside the tunnels, the *Phacelia* crop was split into two plots. Each had a surface of 7.5 m^2 (5 m x 1.5 m). One plots was considered as sheltered area (R, not treated with test item), the other (T) as treated area. Two bumblebee hives and a watering place were placed in each unit.
Exact interior design of the tunnel is shown in the figure below:



R: sheltered area
T: treated area

Application rates: Control (C): Tap water
Treatment rate (T): 0.2 kg a.s./ha during foraging activity

	Reference rate (R): 1.2 L/ha (600 g a.s./ha) The spray volume was 300 to 315 L of spray mixture per hectare. Nozzles were previously tested on a calibration scale in order to check spraying regularity.
Data sampling:	Data for mortality, foraging activity and behaviour of the bees were assessed.
Data analysis:	All data were charted in diagrams comparing bee individuals (dead and foraging bees, respectively) and experimental duration.
Deviations from the study plan:	No deviation mentioned.

Climatic conditions during the experiment:

This experimentation took place over a period with weather and temperatures favourable to foraging. The period before and after the day of product application was warm and dry.

The environmental conditions are shown in the following table:

Table 20: Weather data

	Temperature mini (°C)	Temperature maxi (°C)	Rainfall (mm)
August 22nd 2000	8	25	0
August 23rd 2000	14	30	0
August 24th 2000	14	33	0
August 25th 2000	17	35	0
August 26th 2000	14	25	0
August 27th 2000	15	25	12
August 28th 2000	11	24	0
August 29th 2000	9	25	0
August 30th 2000	14	20	0
August 31st 2000	12	26	4,5
September 1st 2000	14	23	5
September 2nd 2000	14	28	0
September 3rd 2000	12	21	0
September 4th 2000	8	20	0

Pesticide history of the field site:

Only the maintenance of the field site is stated in the study report and is shown in the following table.

Table 21: *Phacelia* crop data

Date	Operation	Caracteristics
May	Soil preparation	Herbicide application and harrowing : seedbed preparation and weed destruction
20/06/00	plot sawing + rolling	Specie *Phacelia tanacetifolia* variety : TITAN Reference : H 6 53 / 373 06 – 1998 OECD system, EC norms, ICTA method Model 3 580.927 584 Dose seed 6,5 kg per ha
22/09/99	Destruction	Crushing the crop on experimental plots

The effects of Deltamethrin EG 6.25 W were tested on the bumblebees (*Bombus terrestris*) under confined semi-field conditions by following the guidance document C.E.B. method no. 129.

The aim of the study was to evaluate potential side effects of a spray application of Deltamethrin EG 6.25 W on the bumblebees (*Bombus terrestris*) under forced exposure conditions. This study included three exposure groups (tunnels) each: one water treated control group, one test-item group and one reference item group. Bumblebee colonies were submitted to test substances while foraging on sprayed crops. The bee colonies were confined in tunnel parts. Two bumblebee boxhives were introduced into each elementary unit 6 days before product applications in order to enable the colonies to adapt to their environment.

Mortality in each tunnel unit was recorded on a daily basis for all areas covered with plastic film, from 5 days before treatment (5DBT) to 7 days after treatment (7DAT). Moreover, the day on which product application was carried out (day 0) additional counts were done at the end of the day (0DAT) in order to establish possible brutal intoxication of foraging bumblebees. The total mortality rate recorded in a tunnel unit for a given day resulted from adding up mortality rates observed in each of the plastic rows in the unit.

Foraging was observed from 2DBT to 3DAT, on all treated and sheltered (untreated) areas. It was possible to adapt the time of counting to the environment of the trial and to active foraging periods. Counts could be shifted if activity was not considered satisfying (late activity due to morning mist or disturbed by rainfall, etc.). This parameter was also taken into account for an additional count on 0DAT, during the hour following product application.

Observations on behaviour were carried out during the trial in order to better understand the incidence of pesticide application on bumblebee behaviour. But these observations appeared especially important on the day the products were applied. On this time and during the thirty minutes following product application, bumblebee reactions and behaviour in each of the tunnels were observed (intense flying, clusters on the net or at the entrance of the box hive, aggressiveness, beginning of intoxication...). In general, this observation phase continued all over the day, between counts, and results were compared to usual activities before product application.

The influence of the test item was evaluated by comparing the results obtained in the test item treatment group to those of the control and the reference item group.

The following endpoints were assessed:

- Number of dead bees per day before as well as after the applications in the control, the test item group and the reference item group, respectively
- Number of foraging bees/m^2 per day on all the areas (treated and sheltered) before as well as after the applications in the control, the test item and the reference item group, respectively
- Behaviour of the bees during assessment in the control, the test item and the reference item group, respectively

Dates of Work: August 22nd to September 4th 2000

Findings:

Mortality

A summary of the daily mortality and total mortality results are shown in the following table.

Table 22: Daily mortality data

Total	Deltamethrin EC 6.25 at 0.2 kg a.s./ ha	Zolone Flo	Water control
5DBT - 23th August	0	0	0
4DBT- 24th August	0	1	0
3DBT- 25th August	1	2	0
2DBT - 26th August	0	0	0
1DBT - 27th August	1	0	2
0DBT - 28th August	0	0	0
0DAT - 28th August	0	0	0
1DAT - 29th August	0	0	0
2DAT - 30th August	0	0	1
3DAT – 31st August	0	0	0
4DAT – 1st September	1	0	1
5DAT – 2nd September	1	0	1
6DAT – 3rd September	0	0	0
7DAT – 4th September	0	1	1

DBT: days before treatment
DAT: days after treatment

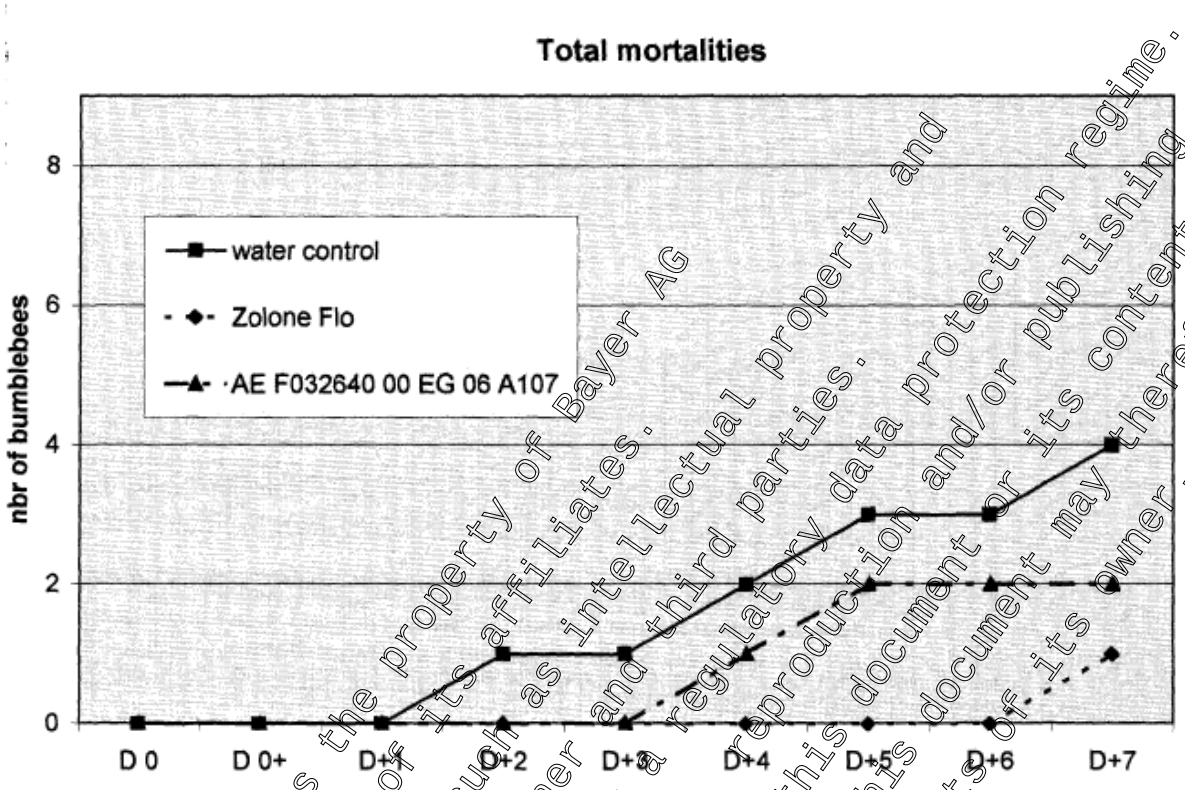


Figure 1: Total mortalities: Deltamethrin EG 6.25 W (AE F032640 00 EG06 A107) at 0.2 kg a.s./ ha, reference item (Zolone flo) and the water control
DO: 0 days before treatment
DO+: 0 days after treatment
D+1 to D+6: 1 to 7 days after treatment

Daily mortality did not increase in any tunnel after treatment. Only one individual was collected per day in the Deltamethrin EG 6.25 tunnel at 4DAT and 5DAT. It was the same as in the reference tunnel where there was no higher mortality. In the control unit (treated with water) the colony was not more disturbed than the Deltamethrin EG 6.25 treatment. Mortality rates recorded varied very few along the week. Looking to total mortalities, curves were similar seven days after treatment (7DAT) in both study item and water control as the graph showed. After a week total mortalities contained between 1 and 4 individuals that meant no impact.
Therefore all the graph curves of the mortalities were increasing: Records were taken into account from the day of application (0DAT) in order to understand the impact of product applications.
There was no toxic reference which might have provided an eventual higher mortality, so Deltamethrin EG 6.25 as well as Zolone Flo was considered as neutral on bumblebees, with data closed to the untreated water control.

Foraging activity

A summary of the daily foraging activity and breaking down of foraging on treatment day results are shown in the following tables.

Table 23: Foraging data: Deltamethrin EW 15 at 0.2 kg a.s./ ha

AEF032640 00 EG06 A107		number of bumble-bees per zone		calculated data	
day	time	R	T	R / m²	T / m²
26 août 00	13h00	13	18	1,7	2,4
D-2	15h00	14	17	1,9	2,3
	16h30	15	20	2,0	2,7
					2,4
27 août 00	10h00	21	24	2,8	3,2
D-1	11h00	31	39	4,1	5,2
	12h00	43	35	5,7	4,7
					4,4
28 août 00	11h00	33	30	4,4	4,0
D 0	11h30	36	44	4,8	5,9
					4,9
	13h00	24	15	3,2	2,0
	15h30	20	44	2,7	5,9
	16h45	38	55	5,1	7,3
					5,1
29 août 00	10h00	25	21	3,3	2,8
D+1	11h00	22	35	2,9	4,5
	12h00	24	23	3,2	3,1
					3,4
30 août 00	10h00	26	30	3,5	4,0
D+2				0,0	0,0
					4,0
31 août 00	10h00	15	22	2,0	2,9
D+3	11h00	18	23	2,4	3,1
	12h00	29	32	3,9	4,3
					3,4

R : number of bumblebees counted on sheltered area by an experimentator
T : number of bumblebees counted on the treated area by an experimentator.
R / m² : number of mean bumblebees per metre square on R (sheltered area)
Precision calculation : R / m² = R / 7.5
Precision calculation : T / m² = T/ 7.5
D-2/ D-1: 1/ 2 days before application
DO: 0 days before application
D+1/ D+2/ D+3: 1/ 2/ 3 days after application

Table 24: Foraging data: Zolone Flo

Zolone Flo		number of bumble-bees per zone		calculated data	
day	time	R	T	R / m²	T / m²
26 août 00	13h00	15	9	2,0	1,2
D-2	15h00	17	10	2,3	1,3
	16h30	14	9	1,9	1,2
					1,2
27 août 00	10h00	20	21	2,7	2,8
D-1	11h00	26	19	3,5	2,5
	12h00	24	20	3,2	2,7
					2,7
28 août 00	11h00	18	21	2,4	2,8
D 0	11h30	29	22	3,9	2,9
					2,9
	13h00	32	17	4,3	2,3
	15h30	33	27	4,4	3,6
	16h45	33	26	4,4	3,5
					3,5
29 août 00	10h00	19	16	2,5	2,1
D+1	11h00	19	21	2,5	2,8
	12h00	15	24	2,0	3,2
					2,7
30 août 00	10h00	36	23	4,8	3,1
D+2				0,0	0,0
				0,0	0,0
					2,1
31 août 00	10h00	18	19	2,4	2,5
D+3	11h00	20	26	2,7	3,5
	12h00	26	30	3,5	4,0
					3,3

R : number of bumblebees counted on sheltered area by an experimentator
T : number of bumblebees counted on the treated area by an experimentator
R / m² : number of mean bumblebees per metre square on R (sheltered area).
Precision calculation : R / m^2 = R / 7,5
Precision calculation : T / m^2 = T/ 7,5
D-2/ D-1 : 1/ 2 days before application
DO: 0 days before application
D+1/ D+2/ D+3: 1/ 2/ 3 days after application

Table 25: Foraging data: Water Control

Water control		number of bumble-bees per zone		calculated data	
day	time	R	T	R / m²	T / m²
26 août 00	13h00	22	17	2,9	2,3
D-2	15h00	19	13	2,5	1,7
	16h30	14	13	1,9	1,7
					1,9
27 août 00	10h00	36	26	4,8	3,5
D-1	11h00	32	28	4,3	3,7
	12h00	23	21	3,1	2,8
					3,3
28 août 00	11h00	34	27	4,5	3,6
D 0	11h30	24	28	3,2	3,7
					3,7
	13h00	36	36	4,8	4,8
	15h30	35	37	4,7	4,9
	16h45	30	44	4,0	5,9
					5,2
29 août 00	10h00	30	26	4,0	3,5
D+1	11h00	45	37	6,0	4,9
	12h00	49	43	6,5	5,7
					4,7
30 août 00	10h00	41	34	5,5	4,5
D+2				0,0	0,0
				0,0	0,0
					4,5
31 août 00	10h00	40	39	5,3	5,2
D+3	11h00	47	26	6,3	3,5
	12h00	53	35	7,1	4,7
					4,4

R : number of bumblebees counted on sheltered area by an experimentator
T : number of bumblebees counted on the treated area by an experimentator
R / m² : number of mean bumblebees per metre square on R (sheltered area)
Precision calculation : R / m² = R / 7,5
Precision calculation : T / m² = T/ 7.5
D-2/ D-1: 1/ 2 days before application
D+1/ D+2/ D+3: 1/ 2/ 3days after application

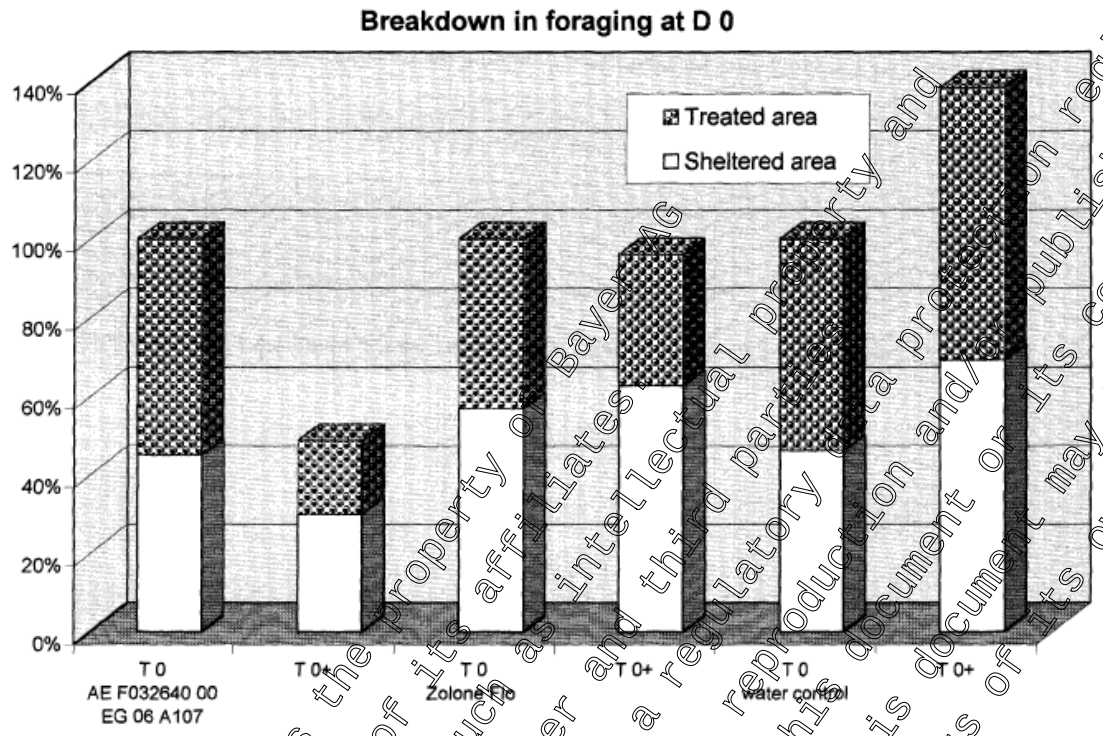


Figure 2: Breakdown in foraging at D 0: Deltamethrin EG 6.25 (AE F032640 00 EG06 A107) at 0.2 kg a.s./ ha, reference item (Zolone flo) and the water control
TO: before product application
TO+: after product application

On day of the product application (day 0), in the morning before treatment (ODBT), foraging was already quite active in the 3 treatment groups and quite similar. The level of this foraging activity was about 3 to 5 bumblebees per m² again.

During the three counts that followed product application, mean foraging trends were a bit different between treatment groups. In fact, foraging activity remained stable in the reference treatment groups where spraying did not disturb the foragers' activity. However, in the unit where Deltamethrin EG 6.25 formulation was applied the activity remained stable as in the untreated treatment groups, and the average level in the afternoon was therefore above 5 bumblebees per metre square. On the same way, the bumblebee colony in the water control treatment groups seemed indifferent to water application and foraging increased during the day over pretreatment phase level.

On the following day (1DAT) foragers' activity decreased in the Deltamethrin EG 6.25 treatment, between 3 and 4 bumblebees per m^2, a medium level between the water control and the standard. Foraging activity decreased too, but slowly in the water control modality, staying over the pre application activity level for the next 2 days. In the modality where the reference item was used this activity did not move and stayed at approximately the same level the day before.

Shortly after product application (0DAT, during the thirty minutes following product application), a repulsive effect was observed in the Deltamethrin EG 6.25 tunnel. The decrease in foraging activity affected both treated and non-treated areas. This confirmed the short term impact of Deltamethrin EG 6.25 on foraging activity on average on the treatment day. On the contrary, the water control modality

showed increasing activity on both sheltered and treated areas, this explained the level over 100 %, while foraging remained stable shortly after treatment in the standard phosalone treatment.

Colony behaviour

In such a test, with homogeneous bumblebee colonies, behaviour was also comparable between the treatment groups, as foraging was quite regular on phacelia plots. Bumblebee foragers only showed little reaction to treatments in the different treatment groups. The volume of a unit treatment group represented sufficient flight space but it was nevertheless confined and colonies adapted to this environment after the first recordings.

From the beginning of this experimental phase, plots were very attractive for foragers and this triggers activity of bumblebees within box hives. During spraying, the bumblebees presented on the experimental plot when the boom passed flew away over treated plot. Generally they came back again a little further away. Experimentators noticed neither any particular aggressiveness nor any frenetic bumbling.

Conclusion:

Overall conditions for conducting this experimental phase of the scheme were favourable to bumblebee activity. Climatic and crop conditions were satisfactory. The different parameters observed agreed with obtained data.

Experimental conditions of the study were quite strict, including confinement and product application carried out during intense foraging activity, on attractive plots.

The use of any phyto-pharmaceutical substance did not give any high mortality stage.

The effects of the test substance Deltamethrin EC 6.25 in the case of this trial on a phacelia crop, only showed a temporary decrease in foraging, but no impact on mortality.

CP 10.3.1.6 Field tests with honeybees

Report:	**KCP 10.3.1.6/01, [redacted]; 2007**
Title:	Assessment of side effects of Deltamethrin EC 25 on the honey bee (*Apis mellifera* L.) in the field
Document No:	M-286584-01-1 (Rep. No.: 20061298/G1-BFEU)
Guidelines:	OEPP/EPPO No. 170 (3), 2001
GLP:	yes

Material and Methods:

The effects of the test substance Deltamethrin EC 25 were tested on the honey bee (*Apis mellifera* L.) under field conditions following the OEPP/EPPO Guideline No. 170 (3). The study comprised one trial which was carried out in Germany. As crop *Phacelia tanacetifolia* was used. In total there were three test fields per trial: one test item field with application of Deltamethrin EC 25, one test item field with application of the reference item Fastac SC and the untreated control field. The distance between the control field (size: 5832 m^2) and the reference item field (size: 5229 m^2) was 2.0 km, the distance between the control and the test item fields T1 (2 smaller fields, separated by approximately 100 m,

sizes: 2592 m^2 and 2898 m^2= total field size 5490 m^2) was >10 km. The distance between the reference item field R1 and the test item field was 12.8 km, respectively. At the each field 4 honey bee colonies were set-up. In the test item field (code: T1) Deltamethrin EC 25 was applied once at an application rate of 7.5 g a.i./ha (nominal). In the reference item field (code: R1) Pastac SC was applied once at a rate of 10 g a.i./ha (nominal). All applications were carried out with a rate of 300 L water/ha on the flowering crop with foraging activity of the bees on the test fields. The control field remained untreated, no application of water was carried out in the control field.

Mortality, flight intensity, and the condition of the colonies and development of the bee brood were assessed before and after application. Homing behaviour was assessed twice before application by marking foraging bees in the crop. As only a small amount of the marked bees were recovered at the hive entrances in an appropriate time-span in all control and treatment groups, detailed observations of the foraging activity in the field and at the hive entrances were conducted instead.

The treatment groups T1 and R1 are individually compared to the flight activity at the control field at the corresponding time of the day. Therefore two subsequent assessments were conducted on the control field (C for T1 and C for R1). This was necessary as the time-lag between the 2 applications was about 90 minutes due to the distance between the test fields. For the evaluation assessments were made at the control field at the same time as the assessments at the reference item or test item field. For the evaluation only data assessed at about the same time for the test item treatment and the control treatment respectively for the reference item treatment and the control treatment were used.

The influence of the test item was evaluated by comparing the results of the test item treatment to the control and reference item data and by comparing the pre- and post-application results of the observations. The following points were assessed:

- Condition of the colonies (strength) and development of the bee brood
- Mortality in the field and in the bee traps in front of the hives
- Foraging activity (number of forager bees/m^2 flowering crop)
- Behaviour of the bees on the crop and around the hive

Findings:

Effects of Deltamethrin EC 25 on the honey bee (Apis mellifera L.) in the field

Test item		Deltamethrin EC 25			
Test species		*Apis mellifera* L., *carnica*			
Exposure		T1 and R1 : spray treatment during foraging activity at full flowering of the crop under field conditions			
Treatment group		Control for T1	Control for R1	T1	R1
Application rate g a.i./ha nominal		-	-	7.5	10.0
Spray volume pro ha [L water/ha]		-	-	300	300
Mean mortality [deadbees/ colony/day]	Pre-application [DAA -4 to 0ba]	24.0		38.3	4.7
	DAA 0ba	18.0		24.5	4.8
	DAA 0aa	9.5		14.5	16.8
	DAA +1	4.3		22.8	5.0
	Post-application [DAA 0aa to +7]	17.4		36.7	9.1
	QM(average):	0.7		1.0	1.9
Daily mean flight intensity [foraging bees/m²]	Pre-application [DAA-4 to 0ba]	2.0	2.3	4.2	7.9
	DAA 0ba	4.2	5.8	6.2	9.4
	DAA 0aa	5.2	5.1	1.1	1.1
	DAA +1	5.0	5.0	5.7	8.1
	Post-application [DAA 0aa to +7]	6.4	6.4	7.8	6.0

DAA = Days after application
ba = before application
aa = after application
$Q_{M(average)}$ = Ø Post-application mortality / Ø pre-application mortality

Observations:

Honey bee mortality

The daily mean bee mortality (number of dead bees in the dead bee traps and in front of the hives) before the application was 38.3 dead bees/colony in the test item group T1, 4.7 dead bees/colony in the test item treatment R1 and 24.0 dead bees in control colonies. In the morning before application on DAA 0 the mean mortality was 24.5 dead bees/colony in the test item group T1, 6.0 dead bees/colony in the test item treatment R1 and 18.0 dead bees per hive in control. In the evening of the application day (DAA 0aa) a mean number of 14.5 dead bees per colony was recorded in the test item group T1, 2.0 dead bees/colony in the reference item treatment R1 and 9.5 dead bees in control colonies. Before the application the natural mortality in the test item treatment and the control was on a higher level than in the reference item treatment. Considering the results before and after application of each treatment group, the mortality in the test item treatment was on the same level during the post-application period as during the preapplication period. The mortality of the reference item treatment was slightly increased after the application. The control mortality remained on about the same level in the pre- and post-application period, but showed a slight increase of mortality between DAA +3 and DAA +5. The value for $Q_{M(average)}$ was calculated as 1.0 in the test item treatment group T1 compared to 0.7 in the control group, indicating that the treatment had no effect on honey bee mortality. In the

reference item treatment the mortality was on a low level during the entire postapplication period and the QM(average) value was 1.9, indicating that the bees were well exposed and the test system was sensitive and adequate for detection of effects by plant protection product on honeybee.

Honey bee flight intensity:
Shortly before application on DAA 0 the mean flight intensity (foraging bees/m²) was 6.2 bees/m² in the test item treatment group T1 and 4.2 bees/m² in the control treatment group for T1. In the reference item treatment group R1 the mean flight intensity was 9.4 bees/m² and 5.8 bees/m² in the corresponding control group for R1. The mean flight intensity pre-application was 4.2 bees/m² in the test item treatment group T1 and 2.0 bees/m² in the control treatment group for T1. The mean flight intensity pre-application in the reference item treatment group R1 was 7.9 bees/m² and 2.3 bees/m² in the control treatment group for R1. During the assessments on DAA 0 after application a decreased flight activity was observed in the test item field as well as in the reference item field and resulted in a mean number of 1.1 foraging bees/m² in the test item treatment group T1 and 1.1 foraging bees/m² in the reference item treatment group R1. In the control group for T1 and in the control group for R1 the mean flight intensity on DAA 0 was 5.2 and 5.1 foraging bees/m² after application respectively.
On the following assessment dates no treatment related difference regarding the flight intensity was observed between the treatment groups. The daily mean flight intensity after the application was 7.4 foraging bees/m² in the treatment group T1, 6.0 foraging bees/m² in the treatment group R1 and 6.4 foraging bees/m² in the control group for T1 and 6.4 foraging bees/m² in the control group for R1. The observation of intense flight and foraging activity of the bees on the test fields was also supported by the fact that the amount of *P. tanacetifolia* pollen in the combs of the colonies of all treatment groups was on a high level (see following chapter).

Condition of the colonies:
Assessments of the colony strength as judged by number of bee ways between combs filled with bees and the brood nest size (number of brood combs per colony) did not indicate significant differences between treatment groups T1, R1 and the corresponding control groups. The colonies of the treatment groups T1 and R1 and control showed all brood stages at the assessment dates during the experimental phase of the study. The colonies of both groups treated with Deltamethrin EC 25 were in good condition throughout the entire observation period, except one colony (2T1) which died due to Varroosis. Although the colonies were checked for Varroa mites before the trial and showed no symptoms of a high infestation, a rapid increase of the mite population is possible to occur in autumn between July and October. The increase can show strong differences between different individual colonies and varies between locations.
Most of the colonies in the treatment groups of the trials showed a high percentage of *P. tanacetifolia* pollen from the total amount of pollen per colony. In the trial the percentage of *P. tanacetifolia* pollen on combs in most of the colonies of the treatments ranged from approximately 20% up to 70% during time of exposure at the test fields. The results of the pollen assessments in the colonies confirms the fact that the bees were actively foraging on the test fields. A quantitative comparison between the results of the treatments is not possible, because the foraging and storage of pollen in a bee colony depends on outside conditions as well as on the individual need of pollen in the bee colony.

Behaviour of the bees during foraging activity:
During the detailed observations of the foraging activity of the bees in the field, symptoms of affected foraging behaviour of the bees, like trembling, shaking or cramping bees, bees showing erratic foraging behaviour, bees hanging or dropping from flowers or green parts of the plant, excessive cleaning or showing other visible impact on behaviour were assessed. In both the test item treatment

and the reference item treatment only a small percentage bees showed symptoms of affected foraging behaviour, and only on the day of application after the application. The fraction and absolute number of honey bees showing affected behaviour was slightly higher in the reference item field compared to the fraction and absolute number of bees foraging in the test item field. On the days following the application no further affected foraging behaviour was observed. No abnormal behaviour was observed in the control during the entire observation period.

Behaviour of the bees at the hive entrance:
During the observations at the hive entrance, symptoms of affected bee behaviour like shaking, trembling or cramping bees, bees showing impaired movements, excessive cleaning behaviour or fighting bees were observed in the test item treatment and the reference item treatment. After the application on DAAO the fraction and absolute number of honey bees showing affected behaviour at the hive entrance was higher in the reference item field compared to the fraction and absolute number of bees at the entrance of hives in the test item field. Before the application, in T1 and also in R1 a very small proportion of bees was assessed which were already showing symptoms which are categorized as affected behaviour. Also in the control group some bees showing abnormal behaviour were noticed in the entire observation period, before and after application of the test item and reference item up to DAA+3. For the evaluation it has to be taken into account that the behaviour of some bees may have been categorized as affected, although abnormal bee-behaviour is not always due to the use of pesticides, and may likewise be triggered by natural or other factors to some extent. A higher degree of affected behaviour in comparison to the control was observed in the entire observation period up to DAA+3 in the test item treatment and in the reference item treatment, was highest on the day of application and showed a decrease on the following days.

Conclusions:
An application of Deltamethrin EC 25 to flowering *Phacelia tanacetifolia* at a rate of 7.5 g a.i./ha (nominal) led to a decrease of the flight intensity on the day of application after the treatment. After the application, the mortality was not elevated and remained below the pre-application level up to DAA +2, increased slightly between DAA +3 and DAA +5 and then returned below the pre-application level. Before the application the mortality was already slightly higher in the test item T1 treatment group and in the reference item group R1 compared to the mortality of the control colony group. In the Deltamethrin EC 25 treatment some bees showed symptoms of affected behaviour at the hive entrance (only in front of the hives) mainly on the day of application after the treatment. In the reference item treatment the fraction of bees showing symptoms of affected behaviour was higher than in the test item treatment and higher than in the control treatment. Symptoms observed and evaluated as affected behaviour at the hive entrance were: bees that were trembling or shaking, bees showing impaired movements, showing intense cleaning behaviour, also bees showing aggressive behaviour and fighting with other bees at the hiveentrance. The condition of the colonies, size of the brood nest and the development of the honey bee brood in the test item treatment group was not different compared to the control during the observation period.

*****

Report:	**KCP 10.3.1.6/02, [redacted]; 2007**
Title:	Assessment of Side Effects of Deltamethrin EC 25 on the Honey Bee (*Apis mellifera* L.) in the Field
Document No:	M-295800-01-1 (Rep. No.: 20071100/G1-BFEU)
Guidelines:	OEPP/EPPO No. 170 (3), 2001
GLP:	yes

The effects of Deltamethrin EC 25 were tested on the honey bee (*Apis mellifera* L.) under field conditions following the OEPP/EPPO Guideline No. 170 (3): Guideline on test methods for evaluation the side-effects of plant protection products on honey bees (OEPP/EPPO, 2001).
The study comprised three trials and was carried out in Germany. Trial G07N001B was carried out in Southern Germany [redacted] trial G07N002B was carried out in Eastern Germany [redacted] trial G07N003B was carried out in Northern Germany [redacted]. As crop *Brassica napus var. napus* was used. In total there were three test fields per trial: one test item field with application of Deltamethrin EC 25, one test item field with application of the reference item Fastac SC and the untreated control field.
For trial G07N001B, the distance between the control field (size: 8,076 m2) and the reference item field (size: 7,800 m2) was 8.4 km, the distance between the control and the test item field (size: 7,600 m2) was 9.3 km. The distance between the reference item field and the test item field the was 2.1 km.
For trial G07N002B, the distance between the control field (size: 111,096 m2) and the reference item field (size: 59,747 m2) was 5.0 km, the distance between the control and the test item field (size: 64,741 m2) was 23.5 km. The distance between the reference item field and the test item field was 28.5 km.
For trial G07N003B, the distance between the reference item field (size: 22,500 m2) and the control field (size: 18,200 m2) was about 13 km, between the test item field (size: 23,040 m2) and the control field the distance was about 9 km. The distance between the test item field and the reference item field was about 8 km.
At each field 4 honey bee colonies were set up. In the test item fields (code: T) Deltamethrin EC 25 was applied once at an application rate of 7.5 g a.i./ha (nominal). In the reference item fields (code: R) Fastac SC was applied once at a rate of 10 g a.i./ha (nominal). All applications were carried out with a rate of 300 L water/ha on the flowering crop with foraging activity of the bees on the test fields. The control field remained untreated, no application of water was carried out in the control field.
Mortality, flight intensity, and the condition of the colonies and development of the bee brood were assessed before and after application. The homing behaviour of forager bees was evaluated by individually marking 10 bees with numbered Opalith-plates and by additionally marking 40 bees with paint once before and three times after application. For each marking date, the behaviour and recovery of 10 Opalith-marked bees and the recovery of 40 paint-marked forager bees were monitored using a special observation hive which allows a quick inspection of the whole colony inside the hive without disturbing the bees. The marked forager bees leaving the hive and returning to the hive were counted once before and three times after application.
The treatment groups T and R are individually compared to the flight activity at the control field at the corresponding time of the day. Therefore, two subsequent assessments had to be conducted on the control field (C for T and C for R) in trial G07N001B. This was necessary in this trial as the time-lag between the 2 applications was here about 75 minutes due to the distance between the test fields and the duration of the application. In the other two trials, one control assessment was sufficient. For the evaluation, assessments were made at the control field at the same time as the assessments at the reference item or test item field. For the evaluation only data assessed at about the same time for the

test item treatment and the control treatment respectively for the reference item treatment and the control treatment were used.
The influence of the test item was evaluated by comparing the results of the test item treatment to the control and reference item data and by comparing the pre- and post-application results of the observations. The following points were assessed:

- Condition of the colonies (strength) and development of the bee brood
- Mortality in the field and in the bee traps in front of the hives
- Foraging activity (number of forager bees/m² flowering crop)
- Behaviour of the bees in the crop and around the hive
- Homing behaviour of forager bees

Findings:

Toxicity to Honey Bees, Field Test

Test item (T)		Deltamethrin EC 25			
Reference item (R)		Fastac SC			
Test object		*Apis mellifera* L. *carnica*			
Exposure		T and R: spray treatment during foraging activity at full flowering of the crop under field conditions			
Trial code/Location		G07N001B / Near Tübingen			
Treatment group		Control for Ta	Control for Ra	Test item	Reference item
Application rate g a.i./ha (nominal)		-		7.5	10.0
Spray volume pro ha [L water/ha]		-	-	300	300
Mean mortality [dead bees/ colony/day]	Pre-application [DAA-2 to 0ba]:	16.0		18.5	24.3
	DAA0ba:	15.5		13.0	29.5
	DAA0aa:	9.0		8.3	10.3
	DAA+1:	10.0		6.5	6.5
	Post-application DAA0aa to +7]:	12.2		10.4	9.8
	QM(average):	0.8		0.6	0.4
Daily mean flight intensity [forager bees/m2]	Pre-application [DAA-2 to 0ba]:	2.3	2.9	2.2	2.4
	DAA0ba:	4.2	3.0	3.8	2.8
	DAA0aa:	2.9	3.4	0.9	0.6
	DAA+1:	3.0	3.0	2.2	1.3
	Post-application [DAA0aa to +7]:	3.0	3.1	2.2	2.7

a = Two separate sets of assessments were conducted on the control field of trial G07N001B (C for T and C for R) on DAA0aa due to a time-lag between the applications of T and R of more than 60 minutes. For the evaluation, only control group data assessed at about the same time as in the test item treatment and the reference item treatment, respectively, were used.

Toxicity to Honey Bees, Field Test (continued)

Trial code/Location		G07N002B / Near Gerichshain		
Treatment group		Control	Test item	Reference item
Application rate g a.i./ha (nominal)		-	7.5	10.0
Spray volume pro ha [L water/ha]		-	300	300
Mean mortality [dead bees/ colony/day]	Pre-application [DAA3 to 0ba]:	41.9	25.2	22.2
	DAA0ba:	17.0	19.8	7.0
	DAA0aa:	15.5	14.0	6.8
	DAA+1:	33.8	21.0	19.0
	Post-application DAA0aa to +7]:	20.3	16.2	17.9
	QM(average):	0.5	0.6	0.8
Daily mean flight intensity [forager bees/m²]	Pre-application [DAA-3 to 0ba]:	1.5	1.6	1.5
	DAA0ba:	1.8	2.2	2.0
	DAA0aa:	1.6	1.1	0.0
	DAA+1:	2.5	1.7	1.7
	Post-application [DAA0aa to +7]:	2.4	1.7	1.6

Trial code/Location		G07N003B / Near Celle		
Treatment group		Control	Test item	Reference item
Application rate g a.i./ha (nominal)		-	7.5	10.0
Spray volume pro ha [L water/ha]		-	300	300
Mean mortality [dead bees/ colony/ day]	Pre-application [DAA-3 to 0ba]:	24.7	18.2	28.0
	DAA0ba	11.5	8.8	17.0
	DAA0aa	6.3	12.8	9.8
	DAA+1	3.3	4.0	6.8
	Post-application DAA0aa to +7]:	5.2	7.6	9.7
	QM(average):	0.2	0.4	0.3
Daily mean flight intensity [forager bees/m²]	Pre-application [DAA-3 to 0ba]:	1.3	1.2	1.8
	DAA0ba	1.8	1.0	2.6
	DAA0aa	2.4	0.2	0.6
	DAA+1	0.5	0.3	0.1
	Post-application [DAA0aa to +7]:	1.8	1.1	1.0

DAA = Days after application
QM(average) = Ø Post-application mortality ÷ Ø pre-application mortality
ba = before application
aa = after application

Observations Trial G07N001B

Honey bee mortality (Trial G07N001B)
The daily mean bee mortality (number of dead bees in the dead bee traps and in front of the hives) before the application was 18.5 dead bees/colony in the test item group, 24.3 dead bees/colony in the reference item treatment and 16.0 dead bees in control colonies. In the morning before application on DAA0 the mean mortality was 13.0 dead bees/colony in the test item group, 29.5 dead bees/colony in the reference item treatment and 15.5 dead bees per hive in control. A mean number of 8.3 dead bees per colony was recorded in the test item group, 10.3 dead bees/colony in the reference item treatment and 9.0 dead bees in control colonies was found on the application day after application (DAA0aa). Before the application the natural mortality in the reference item treatment and the test item treatment was on a slightly higher level than in the control treatment. Considering the results before and after application of each treatment group, the mortality in the test item treatment was on a lower level during the post-application period as during the pre-application period. The mortality of the reference item treatment was also slightly lower after the application. The control mortality remained on about the same low level in the pre- and post-application period.
The value for QM(average) was calculated as 0.6 in the test item treatment group compared to 0.8 in the control group, indicating that the treatment had no effect on honey bee mortality. In the reference item treatment the mortality was on a low level during the entire post-application period and the QM(average) value was 0.4.

Honey bee flight intensity (Trial G07N001B)
Shortly before application on DAA0 the mean flight intensity (forager bees/m2) was 3.8 bees/m2 in the test item treatment group and 4.2 bees/m2 in the control treatment group for T. In the reference item treatment group the mean flight intensity was 2.8 bees/m2 and 3.0 bees/m2 in the corresponding control group for R. The mean flight intensity pre-application was 2.2 bees/m2 in the test item treatment group and 3.3 bees/m2 in the control treatment group for T. The mean flight intensity pre-application in the reference item treatment group was 2.4 bees/m2 and 2.9 bees/m2 in the control treatment group for R. During the assessments on DAA0 after application a decreased flight activity was observed in the test item field as well as in the reference item field and resulted in a mean number of 0.9 forager bees/m2 in the test item treatment group and 0.6 forager bees/m2 in the reference item treatment group. In the control group for T and in the control group for R the mean flight intensity on DAA0 was 2.9 and 3.4 forager bees/m2 after application respectively. On DAA+1 the flight intensity was 3.0 forager bees/m2 in the control treatment group, the flight intensity observed in the treatment groups T and R was slightly lower, 2.2 in the test item treatment group and 1.3 forager bees/m2 in the reference item treatment group.

On the assessment dates following DAA+2 no treatment related difference regarding the flight intensity was observed between the treatment groups.
The daily mean flight intensity after the application in the entire post-application period was 2.2 forager bees/m2 in the treatment group, 2.7 forager bees/m2 in the treatment group R and 3.0 forager bees/m2 in the control group for T and 3.1 forager bees/m2 in the control group for R.
After the application on DAA0 the amount of bees foraging in the test item and reference item field was reduced for several hours after application, slightly reduced on DAA+1 and returned to normal foraging activity on DAA+2. Slightly lower flight intensities in the test item treatment after DAA+1 are presumably due to natural reasons and the condition of the test item fields, as the flight intensity in the test item treatment was already lower than the flight intensity in the reference item treatment and the control treatment before application.

Homing behaviour - marking of forager bees (Trial G07N001B)
During the observation of bee behaviour of 10 Opalith-marked bees in the trial on DAA-1 few bees were observed showing affected behaviour in the observation hive in the control and in the reference item treatment group, whereas no affected behaviour was observed in the test item treatment group. On DAA0 after the application few bees with affected behaviour were observed in the test and reference item treatment groups, and slightly more in the reference item treatment group compared to the test item treatment group. On DAA+1 no affected behaviour was observed in the reference item treatment and in the control group, only in the test item treatment group few bees were showing symptoms of affected behaviour.
No clear effect on the recovery of 10 Opalith-marked and 40 paint-marked bees was observed after the application. During Marking II on DAA0 and Marking III on DAA+2 a lower number of Opalith-marked bees of the reference group compared to the control and the test item treatment was noticed returning to the hive, also the recovery of paint-marked bees of the reference item treatment group on DAA0 was slightly lower than the control but not on DAA+2. On Marking IV on DAA+3 the recovery of Opalith-marked bees in the test item treatment group was lower compared to the control treatment group and compared to the reference item treatment group. The recovery of paint-marked bees was slightly lower in the reference item treatment before the application on marking I on DAA-1 compared to the control and test item treatment group. After the application the recovery of bees marked at Marking II in the test item treatment group, the reference item treatment group and the control treatment group was on a similar level. On DAA+2, Marking III, a slightly lower number of bees was recovered in the test item treatment group compared to the reference item and the control treatment group. On Marking IV the recovery of the paint-marked bees in test item treatment and in the reference item treatment group were lower compared to the control but in the range of natural variability. The observation of the paint-marked bees of Marking I from DAA-1 up to DAA+2 did not indicate any impact on the recovery of the forager bees. The amounts of bees recovered in the test item treatment group were on the same level as the amount of bees observed before the application as well as on the same level with the amount of bees counted in the control treatment and the reference item treatment.

Condition of the colonies (Trial G07N001B)
Assessments of the colony strength as judged by number of bee ways between combs filled with bees and the brood nest size (number of brood combs per colony) did not indicate significant differences between treatment groups T, R and the control. The colonies of the treatment groups T and R and control showed all brood stages at any time at the assessment dates and increased colony size during the experimental phase of the study. The colonies of all treatment groups were in good condition throughout the entire observation period.

Behaviour of the bees at the hive entrance and during foraging activity in the crop (Trial G07N001B)
During the observations of the foraging activity no symptoms of affected behaviour were observed in the control treatment. In the test item treatment on DAA0 a slightly reduced foraging activity was observed. Half an hour after the application of the reference item on DAA0 it was observed that the bees would not land on the flowers, at the hive entrance cleaning bees were seen. One hour after application no further symptoms of affected bee behaviour were observed. On DAA+1 the colonies of all treatment groups were slightly nervous at the hive-entrance which was presumably due to natural reasons as it occurred in all treatment groups. On the following assessment dates no further symptoms of affected bee behaviour, like shaking, trembling or cramping bees, bees showing impaired movements, excessive cleaning behaviour, or fighting bees were observed in any of the treatments.

Observations Trial G07N002B

Honey bee mortality (Trial G07N002B)

The daily mean bee mortality (number of dead bees in the dead bee traps and in front of the hives) before the application was 25.2 dead bees/colony in the test item group, 22.7 dead bees/colony in the reference item treatment and 41.9 dead bees in control colonies. In the morning before application on DAA0 the mean mortality was 19.8 dead bees/colony in the test item group, 7.0 dead bees/colony in the reference item treatment and 17.0 dead bees per hive in control. After application on the application day (DAA0aa) a mean number of 14.0 dead bees per colony was recorded in the test item group, 6.8 dead bees/colony in the reference item treatment and 15.5 dead bees in control colonies. Before the application the natural mortality in the reference item treatment and the test item treatment was on a lower level than in the control treatment. Considering the results before and after application of each treatment group, the mortality in the test item treatment was on a lower level during the post-application period as during the pre-application period. The mortality of the reference item treatment was also on slightly lower level after the application. The post-application control mortality decreased compared to the pre-application level and was on about the same low level as observed in the reference and test item treatment group in the pre- and post-application period. The Mortality in the test and reference item treatment group were not increased after application.

The value for QM(average) was calculated as 0.6 in the test item treatment group compared to 0.5 in the control group, indicating that the treatment had not affected honey bee mortality. In the reference item treatment the mortality was on a low level during the entire post-application period and the QM(average) value was 0.8.

Honey bee flight intensity (Trial G07N002B)

Shortly before application on DAA0 the mean flight intensity (forager bees/m2) was 2.2 bees/m2 in the test item treatment group, in the reference item treatment group the mean flight intensity was 2.0 bees/m2 and 1.8 bees/m2 in the control treatment group. The mean flight intensity pre-application was 1.6 bees/m2 in the test item treatment group. The mean flight intensity pre-application in the reference item treatment group was 1.5 bees/m2 and 1.5 bees/m2 in the control treatment group. During the assessments on DAA0aa after application a slightly decreased flight activity was observed in the test item field, a mean number of 1.1 forager bees/m2 in the test item treatment group was observed. In the reference item treatment group flight activity was discontinued, a mean flight intensity of 0.0 forager bees/m2 was observed. In the control group the mean flight intensity on DAA0aa was 1.6 forager bees/m2. On DAA+1 the mean flight intensity was 2.5 in the control, 1.7 in the test item treatment and 1.7 forager bees/m2 in the reference item treatment.

On the following assessment dates no treatment-related difference regarding the flight intensity was observed between the treatment groups. The flight intensity of the control treatment group increased slightly during the post-application period, the mean flight intensity of the test item and reference item groups was on the same level in the entire pre- and post application period. The daily mean flight intensity in the post-application period was 1.7 forager bees/m2 in the treatment group T, 1.6 forager bees/m2 in the treatment group R and 2.4 forager bees/m2 in the control group.

Homing behaviour - marking of forager bees (Trial G07N002B)

During the observations of behaviour and recovery of 10 Opalith-marked bees and the recovery of 40 paint-marked forager bees using a special observation hive, no symptoms of affected behaviour were detected in the entire observation period.

The results do not indicate any differences in the behaviour or in the recovery of Opalith-marked bees between the different treatment groups and the control. The recovery of the paint-marked bees was on a similar level in the control, test item and reference item treatment group.
During four days of observation of paint-marked bees, no differences between the treatment groups control, test item and reference item were perceived.

Condition of the colonies (Trial G07N002B)
Assessments of the colony strength as judged by number of bee ways between combs filled with bees and the brood nest size (number of brood combs per colony) did not indicate differences between treatment groups T, R and the control. On the first and second brood assessment the colonies of the treatment groups T and R and control showed all brood stages at any time and increased colony size. After the second brood assessment no further swarm prevention was conducted, 3 colonies of the control treatment, 2 of the test item treatment and 1 colony of the reference item treatment had swarmed. In these colonies the colony strength as judged by number of bee ways covered with bees decreased, and a reduction of the brood nest size was observed. The lack of eggs and larvae at the third brood assessment date is due to the natural process and the biological procedure of swarming.
Summing up, the colonies of all treatment groups were in good condition throughout the entire observation period. No treatment-related effects were observed.

Behaviour of the bees at the hive entrance and during foraging activity in the crop (Trial G07N002B)
On DAA0 during the first two hours after application of the test item many bees cleaning themselves were observed at the hive entrance. In the reference item treatment, bees cleaning themselves were observed at the hive-entrance during the first half hour after the application, the foraging activity was discontinued after the application until the next Day, DAA+1. The behaviour of foraging bees and around the hive-entrance was normal in the control treatment during the entire observation period.

Observations Trial G07N003B

Honey bee mortality (Trial G07N003B)
The daily mean bee mortality (number of dead bees in the dead bee traps and in front of the hives) before the application was 18.2 dead bees/colony in the test item group, 28.0 dead bees/colony in the reference item group and 24.7 dead bees in control colonies. In the morning before application on DAA0 the mean mortality was 8.8 dead bees/colony in the test item group, 17.0 dead bees/colony in the reference item treatment and 11.5 dead bees per hive in control. After application on the application day (DAA0aa) a mean number of 12.8 dead bees per colony was recorded in the test item group, 9.8 dead bees/colony in the reference item treatment and 6.3 dead bees in control colonies.
On DAA-3 the natural mortality in the reference item treatment was about the level of the control mortality and on a slightly higher level than in the test item treatment. All treatment groups were about the same level of natural mortality before application. Considering the results before and after application of each treatment group, the mortality in all treatment groups was on a lower level during the post-application period as during the pre-application period and on the level of natural mortality. The mortality was not increased by any of the treatments. Only on DAA+3 a slightly higher but still normal range of mortality in the reference treatment compared to the control and test item treatments was observed.
The value for QM(average) was calculated as 0.4 in the test item treatment group compared to 0.2 in the control group and 0.3 in the reference item group, indicating that the treatment had no effect on honey bee mortality.

Honey bee flight intensity (Trial G07N003B)
Shortly before application on DAA0 the mean flight intensity (forager bees/m2) was 1.0 bees/m2 in the test item treatment group and 1.8 bees/m2 in the control treatment group. In the reference item treatment group the mean flight intensity was 2.6 bees/m2. The mean flight intensity in the pre-application period was 1.2 bees/m2 in the test item treatment group and 1.3 bees/m2 in the control treatment group, the mean flight intensity pre-application in the reference item treatment group was 1.8 bees/m2.
During the assessments on DAA0 after application a decreased flight activity was observed in the test item field as well as in the reference item field and resulted in a mean number of 0.2 forager bees/m2 in the test item treatment group and 0.6 forager bees/m2 in the reference item treatment group, in the control group the mean flight intensity on DAA 0 was 2.4 forager bees/m2 after application.
On DAA+1 and on DAA+2 the flight intensity was only reduced in the reference item treatment, the flight intensity of the test item treatment and of the control treatment were on a similar level. The flight intensities of all treatments recovered to a similar level on DAA+3, the differences in the flight intensity on DAA+4 and DAA+5 were presumably due to natural reasons and the weather conditions at the field sites.
On the following assessment dates no treatment related difference regarding the flight intensity was observed between the treatment groups.
The daily mean flight intensity after the application was 1.1 forager bees/m2 in the treatment group T, 1.0 forager bees/m2 in the treatment group R and 1.8 forager bees/m2 in the control group.

Homing behaviour - marking of forager bees (Trial G07N003B)
The observation of behaviour and recovery of 10 Opalith-marked bees and the recovery of 40 paint-marked forager bees using a special observation hive did not indicate any behavioural differences between the different treatment groups. No bees with symptoms of affected behaviour were observed in any of the treatment groups.
No difference in behaviour of Opalith- marked bees of the test item treatment group, the reference item treatment group and the control group was observed. The recovery of Opalith- marked bees was slightly higher in the test item and the reference item treatment group compared to the control. Only on DAA0 on Marking II the recovery of Paint-marked bees was slightly higher in the control treatment compared to the test item treatment and the reference item treatment, and slightly lower on Marking III and Marking IV on DAA+1 and DAA+2. The results show that the recovery of bees marked on Marking I (DAA-1) was not reduced after the application of the test item or the reference item up to the last observation on DAA+2.

Condition of the colonies (Trial G07N003B)
Assessments of the colony strength as judged by number of bee ways between combs filled with bees and the brood nest size (number of brood combs per colony) did not indicate apparent differences between treatment groups T, R and the control. The colonies of the treatment groups T and control showed all brood stages at any time at the assessment dates and increased or maintained colony size during the experimental phase of the study. The colonies of the reference item treatment increased size slightly as judged by the number of bee ways covered but showed a slight reduction of the brood nest size on the last brood assessment. On the last brood assessment 2 of the colonies had no more eggs and a small brood nest size, which indicates that the colonies had swarmed.

Behaviour of the bees at the hive entrance and during foraging activity in the crop (Trial G07N003B)
During the observations at the hive entrance no symptoms of affected bee behaviour, like shaking, trembling or cramping bees, bees showing impaired movements, excessive cleaning behaviour, or

fighting bees were observed in the control treatment. In the test item treatment one hour after the application individual bees (numbers not exactly quantified) with shaking, spinning and cramping movements were observed around the hive entrance. In the reference item treatment it was observed 45 minutes after the application that single bees did not land on flowers, if they did, they remained sitting on the flowers. Other bees showed normal foraging behaviour. After one hour the foraging behaviour was normal, an intense flight intensity was observed at the hive entrance but almost no foraging activity in the crop.

Conclusions:
An application of Deltamethrin EC 25 to flowering *Brassica napus* at a rate of 7.5 g a.i./ha (nominal) led to a mostly slight decrease of the flight intensity on the day of application after the treatment in all trials. After application of the test item, the mortality was not increased in any of the trials. The observations of bee behaviour of individually Opalith-marked and paint-marked bees did not indicate any disturbance of the homing behaviour. Only a few bees in the observation hives or at the hive-entrances showed symptoms of abnormal behavior after application of the test item. In the reference item treatment the fraction of bees showing symptoms of affected behaviour was slightly higher than in the test item treatment and higher than in the control treatment.
The condition of the colonies, size of the brood nest and the development of the honey bee brood in the test item treatment group was not different compared to the control during the observation period and not affected by any of the treatments.
The application of Deltamethrin EC 25 did not result in adverse effects on the honeybees in the trials reported here.

*****

Report:	**KCP 10.3.1.6/03 [redacted] 2009**
Title:	Assessment of Side Effects of Deltamethrin EC 25 on the Honey Bee (*Apis mellifera* L.) Applied at 17.5 g a.s./ha to *Phacelia tanacetifolia* in the Field in Greece (Evening Application)
Document No:	M-358267-01-1 (Rep. No.: S09-00073)
Guidelines:	OEPP/EPPO No. 170 (3) (2001), modified for the scope of this study
GLP:	yes

Material and methods:
This study was designed to determine the effects of Deltamethrin EC 25 on the honey bee (*Apis mellifera* L.) applied to *Phacelia tanacetifolia* in the field in Greece, in the evening after the period of daily bee flight. This GLP compliant study was conducted following the OEPP/EPPO Guideline No. 170 (3), modified for the scope of this study: Guideline on test methods for evaluation the side-effects of plant protection products on honey bees (OEPP/EPPO, 2001).
The study comprised one trial which was carried out in *Phacelia tanacetifolia* in Greece, consisting of one test item treated field (8015 m^2) and an untreated control field (7845 m^2) at a distance of 1.3 km between both fields. In the test item field (T) Deltamethrin EC 25 was applied to the crop once during flowering of the crop at a rate of 17.5 g a.s./ha in 400 L water/ha in the evening after daily bee flight stopped. A field of untreated *Phacelia tanacetifolia* was used as control.
Six commercial bee colonies were placed in both fields, respectively, before the application in the test item field (T) at full flowering of the crop. To ensure that the bees are exposed to the treatment in the test fields, detailed assessments of foraging activity were done before as well as after the application.

Mortality and foraging activity of the bees was checked over 5 days prior to the application in the test field and followed up over 7 days after the application. The condition of the colonies and the bee brood development were checked once before the application and twice afterwards (up to 29 days after the application).
Potential effects of the test item on the honey bees was evaluated by comparing the results of the test item treatment to those of the control treatment and by comparing the post-application results with the pre-application data. The following points were assessed:

- Condition of the colonies (strength) and development of the bee brood
- Mortality in the field and in the bee traps in front of the hives
- Foraging activity (number of forager bees/m²)
- Behaviour of the bees on the crop and around the hive

Findings:

Toxicity to Honey Bees, Field Test

Test item		Deltamethrin EC 25	
Test object		Apis mellifera	
Exposure		One application of Deltamethrin EC 25 at full flowering of the crop in the evening after daily bee flight stopped.	
Treatment group		Test item Treatment	Control
Application date (25 May 2009, full flowering), application rate g a.s./ha		17.5	-
Spray volume per ha [L water/ha]		400	-
Mean mortality [dead bees/colony/ assessment day]	Pre-appl. [DAA -4 to 0ba]:	19.4	22.6
	Pre-appl. [DAA 0ba]:	21.2	19.2
	Post-appl. [DAA +1]:	19.7	11.7
	Post-appl. [DAA + 1 to + 7]:	16.8	16.6
Mean number dead pupae per colony/assessment day (pre-application, DAA -4 to 0ba)		24.9	11.0
Mean number dead pupae per colony/assessment day (post application, DAA +1 to +7)		5.3	1.8
Mean flight intensity [forager bees/m2]	Pre-appl. [DAA -4 to 0ba]:	7.7	8.1
	Pre-appl. [DAA 0ba]:	8.2	8.4
	Post-appl. [DAA +1]:	1.2	4.5
	Post-appl. [DAA +1 to +7]:	1.1	2.1

0ba = before application
DAA = days after application

Honey bee mortality:
During the pre-application period the mean mortality was 22.6 dead adult bees/colony/day in the control group (C) and 19.4 dead adult bees/colony/day in the test item treated group (T). At the assessment on the day after the application (DAA +1) the mean mortality in the test item treatment group was 19.7 dead adult bees/colony/day and 11.7 dead adult bees/colony/day in the control group. The daily mean post-application mortality (DAA +1 to +7) in the test item treatment group T was 16.8 dead adult bees/colony/day and 16.6 dead adult bees/colony/ day in the control group.
The mean number of dead pupae from DAA -4 to +7 was throughout on a relatively high level in both treatment groups, and was generally higher in the test item hives than in the control hives. However,

this difference cannot be attributed to the test item treatment since the proportion of dead pupae remained on the same level throughout the test from DAA -4 until DAA +7 in both treatment groups. On the linen sheets in the crop area of the test fields, the level of recorded bee mortality after the application (mean number of dead bees per day) was 0.8 in the treatment group T and 0.9 in the control field.

Honey bee flight intensity:
The daily mean flight intensity (forager bees/m²) during the pre-application period was 7.7 in the test item treatment group T and 8.1 in the control group. The mean flight intensity after the application on DAA +1 was reduced in the test item treatment field with 1.2 forager bees/m² in comparison to 4.5 in the control group C, but returned to comparable levels between control and treatment group from DAA 2 onwards. The daily mean post-application (DAA +1 to +7) flight intensity was 1.1 forager bees/m² in the test item treatment and was 2.1 forager bees/m² in the control.

Condition of the colonies and honey bee brood development:
The mean strength of the colonies (mean number of bees per colony) in the test item treatment group and in the control group was 12850 and 13449 bees per hive at the brood assessment before start of exposure on DAA -6/-5. On DAA +7 the mean strength of the colonies was 22276 bees per hive in the test item group and 25229 bees per hive in the control group respectively. On the last assessment on DAA +29 the mean strength of the colonies was 26707 bees per hive in the test item group and 26915 bees per hive in the control group, respectively.
The brood nest size changed only slightly during the observation period and no test item related difference in the development of the brood nest was recorded in the colonies.

On the assessments during exposure in the test fields all colonies in both treatment groups showed all brood stages and a similar development. Only one colony in the test item treatment showed a lack of eggs on DAA +7, caused by loss of the queen during the brood evaluation on DAA -6/-5. On DAA +29 the new queen has already started to lay eggs again.
However, all brood stages in all other colonies of the treatment groups were present at the different assessment dates during the experimental phase of the study which shows that the colonies and the queens were in good condition during the observation period.

Before start of exposure the mean percentage of comb area covered with egg, larval and pupal cells per hive was 2.6, 4.0 and 15.6 % in the test item treatment group hives, and 2.8, 5.0 and 13.1 % in the control hives on DAA -6/-5, respectively. On DAA +7 the mean percentage of comb area covered with egg, larval and pupal cells per hive was 4.4, 2.5 and 11.3 % in the test item treatment group hives and 4.2, 4.4 and 14.1 % in the control hives. At the last assessment on DAA +29 the mean percentage of comb area covered with egg, larval and pupal cells per hives was 3.6, 3.3 and 12.6 % in the test item treatment group hives and 4.6, 4.2 and 12.4 % in the control hives with no differences between both treatment groups. Before start of exposure 22.2, 19.4 and 58.5 % of the comb area per hive was covered by brood, food and empty cells, respectively, in the test item treatment group hives, and 20.9, 28.8 and 50.3 % in the control hives.
At the end of exposure on DAA +7 18.2, 48.3 and 33.7 % of the comb area per hive was covered by brood, food and empty cells, respectively, in the test item treatment group hives, and 22.7, 44.2 and 33.0 % in the control hives. 29 days after start of exposure 19.5, 56.9 and 23.7 % of the comb area per hive was covered by brood, food and empty cells, respectively, in the test item treatment group hives, and 21.1, 50.8 and 28.2 % in the control hives.

Honey bee behaviour in front of the colonies and within the crop
No differences regarding the behaviour of the bees were observed between the test item treatment group T and the control group C.

Conclusion:
The test item treatment did not result in an adverse effect on honey bees as determined by mortality. Only on the day after the application in the previous evening, reduced flight intensity was observed in the test item treated field, which does not constitute an adverse effect. Flight activity returned to comparable levels again on the second day after application. Differences of bee behaviour between control and treatment group were not observed. The condition of the colonies as assessed by colony strength and size of the brood nest was not affected by the treatment. No evidence of an irritation or termination of the development based upon exposure to treated crops was obtained in the colonies of the test item group and the control colonies.
As a conclusion, it can be stated that an application of Deltamethrin EC 25 at a rate of 17.5 g a.s./ha in the evening after daily bee flight in a flowering, bee-attractive crop did not cause any adverse effects to exposed bee colonies under field conditions.

*****

Report:	**KCP 10.3.1.6/04, [redacted]; 1998**
Title:	Assessment of side effects of AE F032640 00 EG06 A106 on the honey bee (*Apis mellifera* L.) in the field following application during bee-flight
Document No:	M_184784-01-1 (C002608)
Guidelines:	BBA VI, 23-1 EPPO 170
GLP:	yes

Material and Methods:
The side effects of the test substance AE F032640 00 EG06 A106 were tested on the honey bee *(Apis mellifera L.)* at three different locations in Germany with different bee material following the Guideline for the testing of crop protection products for registration of the Federal Biological Research Centre for Agriculture and Forestry (BBA), Federal Republic of Germany part VI, 23-1 (STUTE et al. 1991) and the Guideline of the European and Mediterranean Plant Protection Organization No. 170 (EPPO, 1992). The test locations were [redacted] AE F032640 00 EG06 A106 was applied with commercial equipment on fields of flowering *Phacelia tanacetifolia* Benth. under actual use conditions according to the recommendations of the sponsor at an application rate of 7.5 g a.i./ha (corresponding to 122.5 g product/ha). The effect of the application was examined on bee colonies used for honey production, which were placed near the test field.
Colonies of comparable strength located at a field with flowering *Phacelia tanacetifolia* which was not treated were used as control group. The effect of AE F032640 00 EG06 A106 was evaluated by comparing the bees of the test substance variant with the bee hives near the control field in view of the following observations:

- Mortality in front of the bee hives and in the field
- Flight intensity in the field
- Behaviour of the bees at the entrance of the hives
- Condition of the colonies and development of bee brood

Effect on honey bee mortality:
At none of the three test location the application of AE F032640 00 EG06 A106 resulted in an acute intoxication of adult bees. In both replications carried out at test location [redacted] (Trial Code: G98092B) an increase of mortality was observed during the post-application period in the test substance and control variant. In the test substance variant the value for $Q_{M(average)}$, i.e. average post-application mortality divided by the average preapplication mortality, was 1.1 in the 1st replication and 2.1 in the 2nd replication compared to 1.2 and 2.4 in the control variant.

In both replications performed at test location [redacted] (Trial Code: G98093B) the average post-application mortality was lower than the average preapplication mortality. In the test substance variant the value for $Q_{M(average)}$ was 0.3 in the 1st replication and 0.5 in the 2nd replication compared to 0.4 and 0.3 in the control group. At test location [redacted] (Trial Code: G98094B) the determination of Coverage) as 0.2 and 0.9for the test substance variant confirmed that the mortality was not increased during the post-application period in comparison to the pre-application period. In the control variant a slight increase of mortality was observed in both replications ($Q_{M(average)}$ in both replications = 1.2).

Effects on honey bee flight intensity:
In all of the six replications a slight decrease of flight intensity occurred directly after application of AE F032640 00 EG06 A106 and the flight intensity dropped below the level of the control variant. During the post-application period evaluations showed flight intensities at levels which were in the range of results in the control variant.

Effects on honey bee brood development:
Regarding the colonies strength and the bee brood development no abnormal difference which could be attributed to the influence of the test substance were observed between the AE F032640 00 EG06 A106 variant and control.

Conclusion:
According to the results of this study at three test locations in Germany, AE F032640 00 EG06 A106 showed no significant effects on adult honey bees and the development of the bee brood.

*****

Report:	**KCP 10.3.1.6/05, [redacted] 2013**
Title:	Assessment of Side Effects on the Honeybee (*Apis mellifera* L.), Exposed to *Phacelia tanacetifolia*, Sprayed Sequentially with Deltamethrin During Flowering in a Long-Term Field Study in North Alsace, France
Document No:	M-452717-01-1 (Rep. No.: S10-03820)
Guidelines:	OEPP/EPPO Guideline No. 170 (4) (2010), SANCO/3029/99 rev. 4
GLP:	yes

Objective

The objective of this field study was to assess potential effects of the active substance deltamethrin, formulated as Deltamtherin EW 15 on bees, considering acute and chronic effects such as mortality, flight intensity, colony strength, colony health and vitality, brood and food development and overwintering performance.

Material and Methods

Test item: Deltamethrin EW 15B G (spray application product, Batch-ID: 2011-002948)
Test organism: *Apis mellifera* L. (Hymenoptera, Apidae), provided by [redacted], Germany
Crop used for field study: *Phacelia tanacetifolia*
Study dates / location: June 2011 - March 2012, [redacted] France [redacted]
Description field plots: The size of the field plots were approx. 2.11 ha (test item treatment field) and approx. 2.23 ha (control field). The field plots were separated by 4.28 km in order to minimise the chance of the bees from T visiting the field plot of C or vice versa.
The colonies were placed at the field sites early in the morning on 15 Jun 2011 at early to full flowering of *P. tanacetifolia* (C: BBCH 63-64, T: BBCH 64).
Treatments:

- Test item group T: Two applications of test item Deltamethrin EW 15B G (target application rate: 2 x 12.5 g a.s./ha, spray interval 13 days). The applications were performed during flowering of *P. tanacetifolia*. The first application was carried out after set-up of the honeybee colonies at the test fields during flowering of *P. tanacetifolia* on 21 Jun 2011 (BBCH 65). The 2nd application was performed on flowering *P. tanacetifolia* on 04 Jul 2011 (BBCH 65-67). The applications were carried out during honeybee flight. The actual application rate was 13.5 g a.s./ha (1st application) and 13.4 g a.s./ha (2nd application) in the test item group.
- Untreated control C: No application was performed on the corresponding control field plot (C).

Assessments

The effects of honeybee exposure to Deltamethrin EW 15B G-treated *Phacelia tanacetifolia* flowers were examined on six commercial honeybee colonies placed at each test field.
The influence of Deltamethrin EW 15B G was evaluated by comparing the results of the test item group to the data of the control regarding the following observations:

- Total and mean number of dead honeybees
- Flight intensity
- Behaviour of bees in the crop and around hives

- Condition of colonies (number of bees (colony strength), mean values of the different brood stages per colony and assessment date)
- Colony health (bee diseases, bee viruses)
- Residue analysis

Seven days before the first application, the first colony assessment was performed, which included an assessment of the colony strength and the brood and food status. Pollen, nectar and wax from combs, honeybees (for disease and virus analysis), as well as nectar for AFB analysis were sampled on the same day.

At the end of the flowering period at BBCH 69, the honeybee colonies were relocated to a monitoring site without extensive agricultural crops attractive to bees. Here colony health and strength were assessed. Pollen, nectar and bee wax from combs were collected for residue analysis until 22 Mar 2012.
Samplings of honeybees for disease and virus analysis and nectar for AFB analysis were performed twice after relocation of the colonies to the monitoring site.

Findings

Mortality and Flight Intensity

Summary of Effects on Honeybees during the Exposure Phase of the Study

Treatment group		Control (C)	Test item treatment (T)
Daily mean mortality (dead bees/colony) ± STD	Pre-application 1 (5DBA1 to 0DBA1)	24.1 ± 12.8	29.5 ± 31.0
	Post-application 1 (0DAA1 to 0DBA2)	18.9 ± 32.3	10.1 ± 16.2
	Post-application 2 (0DAA2-17DAA2)	8.4 ± 9.6	7.7 ± 10.0
	Post-application total (5DBA1 to 17DAA2)	13.0 ± 23.1	8.7 ± 13.1
Daily mean flight intensity (bees/m²) ± STD	Pre-application 1 (5DBA1 to 0DBA1)	3.1 ± 3.1	6.0 ± 5.8
	Post-application 1 (0DAA1 to 0DBA2)	4.1 ± 2.8	7.6 ± 4.2
	Post-application 2 (0DAA2-17DAA2)	2.1 ± 2.0	2.5 ± 2.7
	Post-application total (5DBA1 to 17DAA2)	3.0 ± 2.6	4.8 ± 4.2

DBAn: days before application (number n); DAAn: days after application (number n)

Mortality of Honeybees

Pre-application phase (5DBA1 to 0DBA1): mortality in test item group slightly higher (mean value: 29.5 dead bees/colony/day) than in control (mean value: 24.1 dead bees/colony/day), but still in the same range for both treatment groups.

After first application of test item:
0DAA1: mean mortality in T (26.2 dead bees/colony/day) moderately higher than in control (8.8 dead bees/colony/day) but still below the mean pre-application mortality in T.

1DAA1: mean mortality in T (11.8 dead bees/colony/day) declined to about the mortality level of control (7.0 dead bees/colony/day).

Entire post-application phase after the 1st application and before the 2nd application (0DAA1 to 0DBA2): mean number of dead bees slightly lower in test item group (10.1 dead bees/colony/day) than in control (18.9 dead bees/colony/day). Mean mortality levels in both treatment groups during this period below the pre-application mortalities.
Calculated mortality quotients during this period: 0.8 in C and 0.3 in T.

After second application of test item:
0DAA2: mean mortality in T (28.3 dead bees/colony/day) was higher than in control (19.0 dead bees/colony/day) but still below mean pre-application mortality in T.
1DAA1: mean mortality in T (9.0 dead bees/colony/day) declined to mortality level of control (9.2 dead bees/colony/day).

Entire post-application phase after the 2nd application (0DDA2 to 17DAA2): mean number of dead bees slightly lower in test item group (7.7 dead bees/colony/day) than in control (8.4 dead bees/colony/day). Mean mortality levels in both treatment groups during this period were below the pre-application mortalities.
Calculated mortality quotients during this period: 0.4 in C and 0.3 in T.

Entire post application phase (0DAA1 to 17DAA2): mortality 13.0 dead bees/colony/day in control and 8.7 dead bees/colony/day in test item group. Calculated mortality quotients for this period: 0.5 in C and 0.3 in T.

Mortality assessment within the crop area:
On linen sheets spread out within the crop area in the test fields, 1.8 dead bees/day were found in the test item field compared to 1.7 dead bees/day in the control during the entire post application phase (0DAA1 to 17DAA2). No notable differences between control and test item group were observed.
Thus, no test item-related adverse effects on mortality were observed.

Flight Intensity

Pre-application phase (5DBA1 to 0DBA1): mean flight intensity in the test fields lower in control than in treatment (3.1 bees/m²/day in C compared to 6.0 bees/m²/day in T).
After first application of test item:
0DAA1: mean flight intensity amounted to 6.4 bees/m²/day in C compared to 4.8 bees/m²/day in T.
1DAA1: mean flight intensity 5.6 bees/m²/day in C compared to 7.5 bees/m²/day in T.
Entire post-application phase after the 1st application and before the 2nd application (0DAA1 to 0DBA2): mean flight intensity 4.1 bees/m²/day in C compared to 7.6 bees/m²/day in T. No notable differences between control and test item treatment group observed during this period.
After second application of test item:
0DAA2: mean flight intensity amounted to 5.5 bees/m²/day in C compared to 3.0 bees/m²/day in T.
1DAA2: mean flight intensity was 4.3 bees/m²/day in C compared to 8.2 bees/m²/day in T.

Entire exposure phase at the field sites after the 2nd application (0DAA2 to 17DAA2): mean flight intensity 2.1 bees/m²/day in C compared to 2.5 bees/m²/day in T. No notable differences between control and test item treatment group observed during this period.
Entire post application phase (0DAA1 to 17DAA2): Total daily mean flight calculated to be 3.0 bees/m²/day in control and 4.8 bees/colony/day in T, respectively.
Thus, no test-item related adverse effects on flight intensity were observed.

Behaviour of the Honeybees

Notable differences in behaviour in the test item group compared to the control group occurred on the day of the first (0DAA1) and the second application (0DAA2). On 0DAA1, up to approx. 700 bees exhibiting intensive cleaning behaviour and up to approx. 80 motionless bees were observed in T. Further observed behavioural differences compared to the control group were observed only in a few bees of the test item group. A slightly elevated number of bees showing intensive cleaning behaviour in T were still present on 1DAA1. On 0DAA2, up to 69 bees were observed in T which exhibited intoxication symptoms (cramping). Further observed behavioural differences affected only a few bees of the test item group.
On all other days during the exposure period, no notable difference in behaviour was observed in the test item treatment group compared to the control group.

Condition of the Colonies

Colony Strength

On the first assessment at 7DBA1 (14 Jun 2011), one day before set-up of the colonies at the test fields, the mean numbers of bees per colony in C and T were 18740 and 13884, respectively. All bee colonies were strong and healthy. The control colonies were slightly stronger than the test item group colonies at the first brood assessment.
On the second assessment on 8DAA1 (29 Jun 2011), the mean number of bees per colony amounted to 16554 bees in C and 13574 bees in T, respectively.
The 3rd colony assessment was performed on the last day of exposure (21 Jul 2011), 17 days after the 2nd application (= EOE). The mean number of bees per colony in C and T was 19824 and 19977, respectively.
From the 3rd to the 5th colony assessment, the colony assessments were on a rather stable level with only slight fluctuations in colony size.
In both groups (C and T), a noticeable decline of the colony size occurred from end of July (mean value of bees per colony: 18154 in C and 21476 bees in T; 28 Jul 2011) until start of overwintering by middle of October 2011 (mean value of bees per colony: 10488 in C and 13159 bees in T; 13 Oct 2011). This decline of the colony size at the end of summer followed the natural course of colony strength development, with a decreasing tendency from late summer to autumn and spring of the following year.
At the end of overwintering on 22 Mar 2012, the mean colony strength was 5361 bees per colony in C and 7185 bees per colony in T.
No test-item related adverse effects on colony strength were observed during the course of the study.

Brood Stages and Overwintering Success

At the first assessment at 7DBA1 (14 Jun 2011), all colonies of the control and the test item treatment group contained brood of all stages. Brood of all stages was also present in all colonies at all further assessments with a few exceptions on single occasions. However, test item group and control were equally affected regarding the sporadic occurrence of missing brood stages.
At the end of overwintering on 22 March 2012, all colonies of the test item group and the control had successfully survived the winter. All brood stages were present in all colonies except for the absence of eggs in the colonies Ce and Cf. However, since the queens were noticed in both colonies, so it was assumed that this was only a temporary gap of egg laying activity, probably due to low temperatures. In colony Tf, the number of brood cells was slightly lower than in the colonies Ta to Te. This could be attributed to the presence of frost damaged brood in this colony.
No notable differences between the test item treatment group and the control were observed.
Overall, no test item-related adverse effect on colony vitality and brood development was observed, which includes queen survival and overwintering performance.

Food Storage

In the colonies of the control group C and the test item treatment group T respectively the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. All colonies of the study showed approximately equal numbers of pollen and nectar storage cells in C and T throughout the entire observation period, respectively.
Thus, no test item-related adverse effects on the food storage behaviour of the exposed colonies were observed.

Bee Diseases Analysis, AFB Assessment

The objective of the bee disease analysis phase was to determine the presence of different pathogens (*Nosema* sp., *Malpighamoeba mellificae, Varroa destructor, Paenibacillus larvae*) in bee samples taken at different time points during the study period.

Nosema sp. spores

Three control colonies (Ca, Cc, Cd) were free of analysable *Nosema* sp. spores at each of the four sampling dates.
In the bee samples taken from the control colonies at start of exposure, only in colony Cf *Nosema* sp. spores were analysed (high infestation level). All other colonies were free of analysable spores.
In the bee samples taken from control colonies at end of exposure the colony Cb had a high infestation level and the colony Ce had a medium infestation level with *Nosema* sp. spores. In the bee samples of the other control colonies no *Nosema* sp. spores were analysed.
In the bee samples taken at start overwintering, no *Nosema* sp. spores were found in any colony except in colony Cf (high infestation level).
In the control bee samples taken at end of overwintering, no *Nosema* sp. spores were analysed in any colony.

In the test item treatment colony Td no *Nosema* sp. spores were analysed in any of the samples taken in 2011 and 2012.
In the bee samples taken at start of exposure from test item treatment colonies, no *Nosema* sp. spores were found.

In the bee samples taken at end of exposure, one test item treatment colony had a low infestation level (Tf) and two test item treatment colonies had a medium infestation level with *Nosema* sp. spores (Tb and Te).
In the samples taken at start of overwintering, no *Nosema* sp. spores were found in any of the test item treatment colonies.
In the samples taken at end of overwintering, test item treatment colony Ta had a low infestation level and test item treatment colony Tc had a high infestation level. In all other colonies no infestation with *Nosema* sp. spores was analysed.

Varroa mites
The highest infestation rate with *Varroa* mites was 10 % in one bee sample taken at end of exposure of the control colonies (colony Cb). In all other bee samples examined the *Varroa* infestation rate was between 0.0 % and 4.4 % in all samples taken from control colonies.
The *Varroa* mite infestation rate never exceeded the 7 % level in the bee samples taken from the test item treatment colonies. The infestation rate varied between 0.0 % and 5.4 % in all samples analysed.

Malpighamoeba mellificae and spores of *Paenibacillus larvae*
No *Malpighamoeba mellificae* and no spores of *Paenibacillus larvae* were found in any of the samples taken in 2011 and 2012 neither in the control nor in the test item treatment colonies.

Overall, no differences in health could be observed between the control and the test item treatment colonies. Thus, no test item-related adverse effects on colony health in terms of bee diseases were observed.

Pollen Source Identification
The pollen from the pollen traps was collected once before the first application (1DBA1), twice before (3DAA1, 6DAA1) and twice after the 2nd application (1DAA2, 3DAA2) in C and T, respectively.
In the control colonies Ca-Cf, the percentage of *Phacelia* pollen collected per colony was 67-97 % on 1DBA1, 97-100 % on 3DAA1, 94-100 % on 6DAA1, 49-96 % on 1DAA2 and 35-95 % on 3DAA2.
In the test item treatment colonies Ta-Tf, the percentage of *Phacelia* pollen collected per colony was 89-99 % on 1DBA1, 88-99 % on 3DAA1, 63-89 % on 6DAA1, 2-17 % on 1DAA2 and 2-31 % on 3DAA2.
Thus, it can be concluded that *Phacelia tanacetifolia* crop under investigation was a significant foraging area of the exposed colonies.

Bee Virus Analysis
The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at different time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (acute bee paralysis virus), CBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), BQCV (black queen cell virus).
The bee viruses ABPV, CBPV, KBV and IAPV were not detected in any of the samples taken at any time point.
DWV was detected in sample Cc of the control group taken at the time point ‘start of exposure phase’, in samples Cc and Cd of the control group taken at the time point ‘end of exposure phase’, and in samples Ca, Cc, Cd, and Ce of the control group, and in samples Ta, Td, and Tf of

the test item group taken at the time point 'start of overwintering' in 2011, and in sample Tf of the test item group taken at the time point 'end of overwintering in 2012.
SBV was detected in all samples of the control group (Ca–Cf) and in all samples of the test item group (Ta–Tf) taken at the time point 'start of exposure phase', and in sample Tc of the test item group taken at the time point 'end of exposure phase' in 2011.
BQCV was detected in samples Ca, Cb, Cc, Ce, and Cf of the control group, and in samples Tb–Tf of the test item group taken at the time point 'start of exposure phase', and in all samples of the control group (Ca–Cf), and as well as in all samples of the test item group (Ta–Tf) taken at the time point 'end of exposure phase' in 2011.
Thus, no test item-related adverse effects on colony health in terms of virus infestation were observed.

Residue Analysis

Samples of *Phacelia* flowers as well as nectar/honey, pollen/bee bread and bee wax collected from hives were analysed. In pollen, nectar, beewax residues of deltamethrin were below the limit of quantitation (LOQ = 10µg/kg). The measured residues in flowers/blossoms were 158 – 468 µg/kg.

The application was done on 4 July 2011 and the first samples were taken 7 days before application (i.e. 28 June 2011) and the end of analytical phase was 29 November 2012. So maximum storage duration for this study was 17 months.

Conclusions

No test item-related adverse effects were observed on mortality and flight intensity in the test field.
No test item-related adverse effects were observed on honeybee health, colony development (including colony strength, colony health, brood and food development of the colonies) as well as on overall colony vitality throughout the entire field exposure period and throughout the entire monitoring period until the end of overwintering in spring 2012.
Moreover, the overwintering performance of the colonies in the test item treatment group was not adversely affected when compared to control performance.

Overall, it can be concluded that exposure of honeybee colonies to *Phacelia tanacetifolia*, sequentially sprayed with Deltamethrin EW 15B G at a target rate of 12.5 g a.s./ha on two occasions during flowering, did neither cause acute, short-term nor long-term adverse effects on mortality, flight intensity, colony strength, colony health and vitality, brood and food development and overwintering performance in the exposed colonies. Behavioural observations indicated a possible short-term correlation between the application of the test item during bee flight activity and an intensive cleaning behaviour in a larger number of exposed honeybees as well as motionless bees and intoxication symptoms in a smaller number of exposed honeybees.

*****

Report:	**KCP 10.3.1.6/06, [redacted] 2013**
Title:	Assessment of Side Effects on the Honeybee (*Apis mellifera* L.), Exposed to *Phacelia tanacetifolia*, Sprayed Sequentially with Deltamethrin During Flowering in a Long-Term Field Study [redacted], France
Document No:	M-452723-01-1 (S10-03824)
Guidelines:	OEPP/EPPO Guideline No. 170 (4) (2010), SANCO/3029/99 rev. 4
GLP:	yes

Objective

The objective of this field study was to assess potential effects of the active substance deltamethrin, formulated as Deltamethrin EW 15 on bees, considering acute and chronic effects such as mortality, flight intensity, colony strength, colony health and vitality, brood and food development and overwintering performance.

Material and Methods

Test item: Deltamethrin EW 15B G (spray application product, Batch-ID: 2011-002948)

Test organism: *Apis mellifera* L. (Hymenoptera, Apidae), provided by [redacted], Germany

Crop used for field study: *Phacelia tanacetifolia*

Study dates / location: June 2011 - March 2012, [redacted] France [redacted]

Description field plots: The size of the field plots were approx. 2.35 ha (test item treatment field) and approximately 2.25 ha (control field). The field plots were separated by 4.0 km in order to minimise the chance of the bees from T visiting the field plot of C or vice versa.

The colonies were placed at the field sites early in the morning on 10 Jun 2011 at early flowering of *P. tanacetifolia* (BBCH 63).

Treatments:

- Test item group T: Two applications of test item Deltamethrin EW 15B G (target application rate: 2 x 12.5 g a.s./ha, spray interval 13 days). The applications were performed during flowering of *P. tanacetifolia*. The first application was carried out after set-up of the honeybee colonies at the test fields during flowering of *P. tanacetifolia* on 15 Jun 2011 (BBCH 64 - 65). The 2nd application was performed on flowering *P. tanacetifolia* on 28 Jun 2011 (BBCH 65-67). The applications were carried out during honeybee flight. The actual application rate was 16.6 g a.s./ha (1st application) and 12.8 g a.s./ha (2nd application) in the test item group.

- Untreated control C: No application was performed on the corresponding control field plot (C).

Assessments

The effects of honeybee exposure to Deltamethrin EW 15B G-treated *Phacelia tanacetifolia* flowers were examined on six commercial honeybee colonies placed at each test field.
The influence of Deltamethrin EW 15B G was evaluated by comparing the results of the test item group to the data of the control regarding the following observations:

- Total and mean number of dead honeybees
- Flight intensity
- Behaviour of bees in the crop and around hives
- Condition of colonies (number of bees (colony strength), mean values of the different brood stages per colony and assessment date)
- Colony health (bee diseases, bee viruses)
- Residue analysis

Six days before the first application, the first colony assessment was performed, which included an assessment of the colony strength and the brood and food status. Pollen, nectar and wax from combs, honeybees (for disease and virus analysis), as well as nectar for AFB analysis were sampled on the same day.

At the end of the flowering period at BBCH 67 - 69, the honeybee colonies were relocated to a monitoring site without extensive agricultural crops attractive to bees. Here colony health and strength were assessed. Pollen, nectar and bee wax from combs were collected for residue analysis until 23 Mar 2012.
Samplings of honeybees for disease and virus analysis and nectar for AFB analysis were performed twice after relocation of the colonies to the monitoring site.

Findings
Mortality and Flight Intensity

Summary of Effects on Honeybees during the Exposure Phase of the Study

Treatment group		Control (C)	Test item treatment (T)
Daily mean mortality (dead bees/colony) ± STD	Pre-application 1 (5DBA1 to 0DBA1)	15.3 ± 8.3	22.9 ± 13.5
	Post-application 1 (0DAA1 to 0DBA2)	11.0 ± 8.5	19.2 ± 27.2
	Post-application 2 (0DAA2-16DAA2)	18.7 ± 32.4	11.8 ± 8.0
	Post-application total (5DBA1 to 16DAA2)	15.2 ± 24.9	15.2 ± 19.5
Daily mean flight intensity (bees/m²) ± STD	Pre-application 1 (5DBA1 to 0DBA1)	4.0 ± 2.5	4.8 ± 2.8
	Post-application 1 (0DAA1 to 0DBA2)	3.4 ± 2.0	5.9 ± 4.1
	Post-application 2 (0DAA2-16DAA2)	1.4 ± 1.4	2.6 ± 2.2
	Post-application total (5DBA1 to 16DAA2)	2.3 ± 2.0	4.1 ± 3.6

DBAn: days before application (number n); DAAn: days after application (number n)

Mortality of Honeybees

Pre-application phase (5DBA1 to 0DBA1): mortality in test item group slightly higher (mean value: 22.9 dead bees/colony/day) than in control (mean value: 15.3 dead bees/colony/day), but still in the same range in both treatment groups.

After first application of test item:
0DAA1: mortality in T (14.5 dead bees/colony/day) was on the same level as in control (11.8 dead bees/colony/day) and below the mean pre-application mortality in T.
1DAA1: mean mortalities were low and amounted to 3.3 dead bees/colony/day in C and to 7.0 dead bees/colony/day in T, and showed no notable differences between the test item treatment colonies and the control colonies.
Entire post-application phase after the 1st application and before the 2nd application (0DAA1 to 0DBA2): mean number of dead bees slightly higher in the test item group (19.3 dead bees/colony/day) than in control (11.0 dead bees/colony/day), but still below the mean pre-application mortality in T. Calculated mortality quotients during this period: 0.7 in C and 0.8 in T.

After second application of test item:
0DAA2: mean mortality in T (15.2 dead bees/colony/day) slightly higher than in control (4.3 dead bees/colony/day) but still on a normal level and below the mean pre-application mortality in T.
1DAA1: mean mortality in T (12.7 dead bees/colony/day) declined to the mortality level of the control (9.5 dead bees/colony/day).

Entire post-application phase after the 2nd application (0DAA2 to 16DAA2):
mean number of dead bees was slightly lower in the test item group (11.8 dead bees/colony/day) than in control (18.7 dead bees/colony/day). The mean mortality levels in the test item group during this period were below the mean pre-application mortality in T. Calculated mortality quotients during this period: 1.2 in C and 0.5 in T.

Entire post application phase (0DAA1 to 16DAA2): mean daily mortality per colony 15.2 dead bees/colony/day in control as well as in test item group. Calculated mortality quotients for this period: 1.0 in C and 0.7 in T.

Mortality assessment within the crop area:
On linen sheets spread out within the crop area in the test fields, 1 dead bees/day were found in the test item field compared to 0.6 dead bees/day in the control during the entire post application phase (0DAA1 to 16DAA2). No notable differences between control and test item group were observed.
Thus, no test item-related adverse effects on mortality were observed.

Flight Intensity

Pre-application phase (5DBA1 to 0DBA1): mean flight intensity on the same level in test fields and in control (4.0 bees/m^2/day in C compared to 4.8 bees/m^2/day in T).
After first application of test item:
0DAA1: mean flight intensity amounted to 6.0 bees/m^2/day in C compared to 1.9 bees/m^2/day in T.
1DAA1: mean flight intensity 2.9 bees/m^2/day in C compared to 1.6 bees/m^2/day in T.

Entire post-application phase after the 1st application and before the 2nd application (0DAA1 to 0DBA2): mean flight intensity 3.4 bees/m²/day in C compared to 5.9 bees/m²/day in T. Besides a slight reduction of flight intensity immediately after the application of the test item on 0DAA1, no notable differences between control and test item treatment group observed during this period.
After second application of test item:
0DAA2: mean flight intensity amounted to 3.8 bees/m²/day in both, C and T.
Entire exposure phase at the field sites after the 2nd application (0DAA2 to 16DAA2): mean flight intensity 1.4 bees/m²/day in C compared to 2.6 bees/m²/day in T. No notable differences between control and test item treatment group were observed during this period.
Entire post application phase (0DAA1 to 16DAA2): Total daily mean flight calculated to be 2.3 bees/m²/day in control and 4.1 bees/colony/day in T, respectively.
Thus, no test-item related adverse effects on flight intensity were observed.

Behaviour of the Honeybees

Notable differences in behaviour in the test item group compared to the control group occurred on the day of the first (0DAA1) and the second application (0DAA2). On 0DAA1, up to approx. 360 bees in total exhibiting intensive cleaning behaviour, up to approx. 20 motionless bees per colony and up to approx. 20 bees in total with intoxication symptoms were observed in T. A slightly elevated number of bees showing intensive cleaning behaviour in T were still present on 1DAA1. Further observed behavioural differences compared to the control group were observed on 6DAA1, 8DAA1, 9DAA1 and 11DAA1, but only a few bees of the test item group were involved. On 0DAA2, up to approx. 300 bees per colony in T exhibited intensive cleaning behaviour and up to 50 motionless bees per colony were observed. Further observed behavioural differences compared to the control group were observed only in a few bees of the test item group. From 1DAA2 until the end of exposure, no notable difference in behaviour was observed in the test item treatment group compared to the control group.

Condition of the Colonies

Colony Strength

On the first assessment on 6DBA1 (09 Jun 2011), one day before set-up of the colonies at the test fields, the mean numbers of bees per colony in C and T were 18365 and 16970, respectively, and were therefore on the same level. On the second assessment on 7DAA1 (22 Jun 2011), the mean number of bees per colony had increased in both treatment groups and amounted to 19237 in C and 19414 in T, respectively. The 3rd colony assessment (last assessment at the field sites) was performed on 16DAA2 (14 Jul 2011). The mean number of bees per colony in C and T was 23012 and 18112, respectively. The lower mean number of bees in T compared to C was most likely due to swarming activity of colony T1. The number of bees of all other test item group colonies increased or remained stable during the period from the 2nd to the 3rd colony assessment. From the 3rd to the 5th colony assessment, the colony assessments were on a rather stable level with only slight fluctuations in colony size.
In both groups (C and T), a noticeable decline of the colony size occurred from beginning of August (mean value of bees per colony: 21913 in C and 19173 bees in T; 04 Aug 2011) until start of overwintering by middle of October 2011 (mean value of bees per colony: 10375 in C and 7841 bees in T; 07 Oct 2011). This decline of the colony size at the end of summer followed the natural course of colony strength development, with a decreasing tendency from late summer to autumn and spring of the following year.
At the end of overwintering on 23 Mar 2012, the mean colony strength was 5050 bees per colony in C and 3068 bees per colony in T.

No test-item related adverse effects on colony strength were observed during the course of the study

Brood Stages and Overwintering Success

At the first assessment at 6DBA1 (09 Jun 2011), all colonies of the control and the test item treatment group contained brood of all stages. Brood of all stages was also present in all colonies at all further assessments with a few exceptions on single occasions. However, test item group and control were equally affected regarding the sporadic occurrence of missing brood stages.
At the end of overwintering on 23 March 2012, all colonies of the test item group and the control had successfully survived the winter. All brood stages were present in all colonies, with the exception of colony Tc, which contained larvae and pupae but no eggs. However, since the queen was noticed in this colony, it was assumed that this was only a temporary gap of egg laying activity, probably due to low temperatures. In colony Tc, the number of brood cells was slightly lower than in the other colonies of the test item group. This could be attributed to the presence of frozen brood in this colony.
No notable differences between the test item treatment group and the control were observed.
Overall, no test item-related adverse effect on colony vitality and brood development was observed, which includes queen survival and overwintering performance.

Food Storage

In the colonies of the control group C and the test item treatment group T, respectively, the natural and typical changes and fluctuations in the relative amount of nectar and pollen storage cells occurred during the observation period. All colonies of the study showed approximately equal numbers of pollen and nectar storage cells in C and T throughout the entire observation period, respectively.
Thus, no test item-related adverse effects on the food storage behaviour of the exposed colonies were observed.

Bee Diseases Analysis (AFB Assessment

The objective of the bee disease analysis phase was to determine the presence of different pathogens (*Nosema* sp., *Malpighamoeba mellificae, Varroa destructor, Paenibacillus larvae*) in bee samples taken at different time points during the study period.

Nosema sp. spores

From the bee samples taken from the control colonies, only in the colonies Cc and Cd *Nosema* spec. spores were analysed. Control colony Cd showed a low infestation level with *Nosema* spec. spores in the bee sample taken at start of exposure and control colony Cc showed a high infestation level in the bee sample taken at end of overwintering. After overwintering, no samples could be analysed for colonies Ce and Cf.
In the bee samples taken at start of exposure in the test item treatment colonies, no *Nosema* spec. spores were found.
The amount of infestations with *Nosema* spec. spores increased moderately in the bee samples of the test item treatment colonies taken at end of exposure. In these samples, test item treatment colony Tb had a low infestation level with *Nosema* spec. spores and test item treatment colonies Ta, Tc, Td and Te had a medium infestation rate, whereas colony Tf was free of analysable *Nosema* spec. spores.
In the bee samples taken at start of overwintering, only test item treatment colony Te had an infestation with *Nosema* spec. spores (medium level).

In the bee samples taken at end of overwintering, *Nosema* spec. spores were found in the test item treatment colonies Tb (medium infestation level) and Tc (high infestation level). No samples were available from end of overwintering for test item treatment colonies Td and Te. In the summer samples, the amount of positive *Nosema* spec. spore findings in the test item treatment colony group was slightly higher than in the control colony group, but the infestation level was not higher than medium. For the health status evaluation, the more distinctive high infestation level occurred once in the control and in the test item treatment group, respectively.

Varroa mites

In three out of 22 bee samples taken from control colonies, *Varroa* mites were found. The infestation rates with *Varroa* mites of these three findings were between 0.4 % and 0.5 % in all samples taken from control colonies. The *Varroa* mite infestation varied between 0.0 % and 4.3 % in all samples analysed.

Malpighamoeba mellificae and spores of *Paenibacillus larvae*

No *Malpighamoeba mellificae* and no spores of *Paenibacillus larvae* were found in any of the samples taken in 2011 and 2012 neither in the control nor in the test item treatment colonies.

Overall, no differences in health could be observed between the control and the test item treatment colonies. Thus, no test item-related adverse effects on colony health in terms of bee diseases were observed.

Pollen Source Identification

The pollen from the pollen traps was collected once before the first application (1DBA1), twice before (1DAA1, 5DAA1) and twice after (7DAA2, 8DAA2) the 2nd application in C and T, respectively.
In the control field, the percentage of *Phacelia* pollen collected per colony was 1-10 % on 1DBA1, 1-7 % on 1DAA1, 72-91 % on 5DAA1, <1-30 % on 7DAA2 and <1-47 % on 8DAA2 in the colonies Ca-Cf.
In the test item treatment field, the percentage of *Phacelia* pollen collected per colony was 10-91% on 1DBA1, 21-71 % on 1DAA1, 94-100 % on 5DAA1, 18-60 % on 7DAA2 and 4-56 % on 8DAA2 in the colonies Ta-Tf.
Thus, it can be concluded that the *Phacelia tanacetifolia* crop under investigation was a significant foraging area of the exposed colonies.

Bee Virus Analysis

The objective of the bee virus analysis was to determine the following bee viruses in bee samples collected at different time points of the year: DWV (deformed wing virus), SBV (sacbrood virus), ABPV (acute bee paralysis virus), CBPV (chronic bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), BQCV (black queen cell virus).
The bee viruses ABPV, CBPV, KBV and IAPV were not detected in any of the samples taken at any time point.
DWV was detected in sample Tc of the test item group taken at the time point ‘start of exposure phase’, in sample Cf of the control group, and in samples Ta and Tc-Tf of the test item group taken at the time point ’end of exposure phase‘, in samples Cc and Cf of the control group, and in samples Ta, Tb, Td, and Te of the test item group taken at the time point ’start of overwintering‘ in 2011, and in samples Ta and Tc of the test item group taken at the time point ’end of overwintering’ in 2012.

SBV was detected in Cc and Cd of the control group, and in samples Ta, Tb, Td, and Tf of the test item group taken at the time point 'start of exposure phase', in samples Ca-Ce of the control group, and in samples Ta and Td of the test item group taken at the time point 'end of exposure phase' taken in 2011.
BQCV was detected in samples Cb-Cf of the control group, and in all samples of the test item group (Ta-Tf) taken at the time point 'start of exposure phase', and in samples Ca, Cc, and Cd of the control group, and in samples Ta, Tb, Te, and Tf of the test item group taken at the time point 'end of exposure phase' in 2011.
Since the bee viruses DWV, SBV and BQCV were detected in both C and T, respectively, no test item-related adverse effects on colony health in terms of virus infestation were observed.

Residue Analysis

Samples of *Phacelia* flowers as well as nectar/honey, pollen/bee bread and bee wax collected from hives were analysed. In pollen and nectar, residues of deltamethrin were below the limit of quantitation (LOQ = 10µg/kg). In beewax the measured residues of the test substance ranged between the LOQ and 22. The measured residues in flowers/blossoms were 68 - 417 µg/kg.

The application done 15 June 2011 and first samples were taken 6 days before application (i.e. 9 June 2011). End of analytical phase 6 February 2013. So maximum storage duration for this study was 20 months.

Conclusions

No test item-related adverse effects were observed on mortality and flight intensity in the test field.
No test item-related adverse effects were observed on honeybee health, colony development (including colony strength, colony health, brood and food development of the colonies) as well as on overall colony vitality throughout the entire field exposure period and throughout the entire monitoring period until the end of overwintering in spring 2012.
Moreover, the overwintering performance of the colonies in the test item treatment group was not adversely affected when compared to control performance.

Overall, it can be concluded that exposure of honeybee colonies to *Phacelia tanacetifolia*, sequentially sprayed with Deltamethrin EW 15B G at a target rate of 12.5 g a.s./ha on two occasions during flowering, did neither cause acute, short-term nor long-term adverse effects on mortality, flight intensity, colony strength, colony health and vitality, brood and food development and overwintering performance in the exposed colonies. Behavioural observations indicated a possible short-term correlation between the application of the test item during bee flight activity and an intensive cleaning behaviour in a larger number of exposed honeybees as well as motionless bees and intoxication symptoms in a smaller number of exposed honeybees.

*****

Report:	**KCP 10.3.1.6/07, [redacted] (1983)**
Title:	Synthesis of works carried out on bees under natural conditions with deltamethrin at international level: Effects on the environment
Document No:	M-151220-01-1 (Rep. No.: RA-83-21-09/A)
Guidelines:	No particular guidelines, special test design
GLP:	no

The report summarised among others a five year study with deltamethrin on bees under natural conditions has been carried out using a new methodology. From 1978 to 1982, 24 trials were carried out with different rates of deltamethrin, water, parathion and phosalone. In this context, however, emphasis is placed on the results of the years 1980 and 1981 obtained with deltamethrin applied at a rate of ≥ 17.5 g a.s./ha.

Materials and methods:
Deltamethrin was applied onto flowering mustard during bee flight (between 1 and 2 p.m.) at the following application rates:
1980: 7.5 g a.s./ha, 10 g a.s./ha, 12.5 g a.s./ha, 12.5 g a.s./ha and 17.5 g a.s./ha
1981: 21.2 g a.s./ha and 35 g a.s./ha
The trials were conducted at [redacted] France) in an environment which was not or only slightly attractive for honey bees (surrounding field crops: maize, beetroot, cereals, potatoes). In this field study white mustard was sown on 1,500 m² plots every 15 to 20 days starting from the end of March. Each time the 1,500 m² consisted of 30 strips (50 m x 1 m) separated by a 0.7 m wide vegetation free and tamped down alley, from which the insects for the mortality assessments were collected later. Each 1,500 m² area was only used for one trial and then it was ploughed. Honey bees of Italian race x Caucasian race hybrids (*Apis mellifera ligustica* x *Apis mellifera caucasia*) from movable frame hives, supplied by a professional bee-keeper, were used. During each trial (and therefore for each application rate), approx. 4 colonies were placed close to the mustard plot The bees from 3 of these hives remained in the trial area for about 20 days, the 4th hive remained at the field station as a control colony the whole trial period.
Two assessments of mortality and honey bee behaviour were made daily at 8 am and 5 pm at the hives. Moreover, each time, the bee-keeper was on the station, he made observations on the behaviour and activity of the brood-comb. A pollen trap was placed on some of the hives.
The number of foraging bees was assessed on 3 (out of 30) randomly selected strips of crop in each trial. Foraging activity was assessed 4 times per day at 10 am, 12 am, 2 pm and 4 pm on the days before the application. On the application day assessments were conducted at 10 am and 12 am before the application and 15 minutes, 1 hour and 3 hours after the application. After the application daily assessments were conducted at 10 am, 12 am, 2 pm and 4 pm. The behaviour of foraging honey bees was assessed.
The number of dead honey bees was determined on the alleys surrounding the 3 selected strips.

Findings:
Observations on mortality:
At a rate of 17.5 g a.s./ha a slight mortality was revealed which, although not high, was still higher than the one observed at 12.5 g a.s./ha. At 21.2 and 35 g a.s./ha marked mortality occurred.

Behaviour of the bees:
No changes in the behaviour of the bees was observed at the hives, moreover, the bees during the trials in the summer showed normal behaviour at the end of the following winter, they recovered a good activity, a normal brood comb etc. On the crop, the behaviour of the foraging bees was normal after application rates of 5 and 12.5 g a.s./ha. At 17.5 g a.s./ha, 21.2 and 35 g a.s./ha some bees crawled on the flowers and showed signs of "sluggishness" which did not last more than one hour after application. However, it was noted that the bees during the sluggishness period were able to fly when touched.

Repellent effects:
At the rates of 7.5 to 17.5 g a.s./ha bees flew away immediately and rapidly behind the spray boom. This behaviour was not observed with lower rates or after a water treatment. The visiting frequency decreased for 2-3 hours after insecticide application. This effect, which can be considered repellent, involved no change in pollination of the visited crop and seemed specific to deltamethrin, since it was not observed either with parathion or with phosalone.

Conclusion:
It is stated that under the tested conditions, deltamethrin applied directly to foraging bees is not hazardous to bees up to 21.2 g a.s./ha.

*****

Report:	**KCP 10.3.1.6/08, [redacted] (1993)**
Title:	Deltamethrin: Safety to Honey Bees
Document No:	M-151216-01-1 (Rep. No.: A72917)
Guidelines:	No
GLP:	No

The report summarised the results of more than 15 years research on bees exposed to deltamethrin (1976 – 1993), using a variety of testing methods and designs. The testing programme started with tests performed in small cages, followed by field tests using large plots to finally come out to trials carried out under plastic greenhouses (tunnels). Roussel Uclaf together with various French scientists was much involved in the development of this methodology. The assessments carried out during these trials established the following results:

- No abnormal mortality up to 17.5 g a.s./ha.
- Clear repellent effect occurring a few minutes after the application and disappearing within less than 24 hours with dose rates of 7.5 g/ha and higher. Bees are flying back to the hives.
- Behavioural (lethargy) of the bees starting at 17.5 g/ha and higher.
- No adverse effect on the quantity and quality of the brood combs, the density of the populations of the hives exposed to the application and the yield of honey.
- Extremely low levels of residues in the honey.
- Absence of residues of deltamethrin in the pollen.

*****

Report:	**KCP 10.3.1.6/09, [redacted] (1987)**
Title:	Decis EC innocuity towards bees: Control of ear aphids in cereals; Control of *Ceuthorrhynchus assimilis payk* on rape.
Document No:	M-151219-01-1
Guidelines:	No
GLP:	No

The study reviews 8 years of experimentation (1977 – 1985) undertaken with deltamethrin in several field trials in several countries in order to determine the effects on bees. In this context, only the test results with application rates ≥ 17.5 g a.s./ha are presented. Trials carried out by Roussel Uclaf in France from 1978 – 1982 showed that Decis did not affect foraging up to a dose of 17.5 g a.s./ha, slight mortality was found at 21.2 g a.s./ha and foraging activity was reduced at 17.5 g a.s./ha. Furthermore, numerous trials were carried out by third parties under semi-natural or natural conditions. For example, in 1983, 8 tests were carried out in Germany under a tent in the open field on *Phacelia*, with testing doses from 15 to 25 g a.s./ha. It was concluded that deltamethrin is non-toxic to bees because in respect to foraging activity and mortality, there was no difference between deltamethrin and non-toxic reference and control. There were also no effects on larval stages in 3-day and 8-day trials. The brood-comb was also unaffected at 8 days.
In 1985, 4 additional studies were carried out in Germany testing 25 g a.s./ha. No differences were found compared to the control at the hive or in the field regarding mortality, foraging activity and hive observation and no long-term effects at up to 4 weeks after treatment.
Thus, the authors concluded that the limit beyond which the first slight signs of toxicity occurred is around 21.2 g a.s./ha.

*****

Report:	**KCP 10.3.1.6/10, [redacted] (1982)**
Title:	Incidence de traitements a la Deltamethrin (Decis FLW) et au Decis B sur l'abeille en conditions naturelles tests station.
Document No:	M-149774-01-2
Guidelines:	No
GLP:	No

The report describes three field studies that were conducted with deltamethrin formulations in the year 1982.

Material and methods:
Test item: Decamethrin (active ingredient: deltamethrin), was applied onto flowering mustard during bee flight (between 1 and 2 pm) with a sprayer (400 L/ha, 4 bar) in trial 1 (7.5 g a.s./ha), trial 2 (25 g a.s./ha) and 3 (deltamethrin & heptenophos). Trial 2 was conducted with Decis Flow 25 g/L at an application rate of 25 g a.s./ha and is summarised below. The study was conducted [redacted] France) in an environment not or only slightly attractive for honey bees. In this field study white mustard was sown on 1,500 m² plots every 15 to 20 days starting from March. This 1,500 m² consisted of 30 strips (50 m x 1 m) separated by a 0.7 m wide vegetation free and tamped down alley, from which the insects for the mortality assessments were collected later. Each 1,500 m² area was only used for one trial and then it was ploughed. Honey bees of Italian race x Caucasian race hybrids (*Apis mellifera ligustica* × *Apis mellifera caucasia*) from movable frame hives, supplied by a

professional bee-keeper, were used. During each trial (and therefore for each application rate), approx. 4 colonies were placed close to the mustard plot for each trial, except for trial 1 where only 2 colonies were used. One control colony remained at the field station.
Two assessments of mortality and honey bee behaviour were made daily at 8 am and 5 pm. On the application day additional mortality assessments were conducted immediately after the application and within one and three hours after the application.
Pollen traps were installed at the hives for the trials 1 and 5. The pollen was taken from the traps daily and weighed to have a measure of the foraging activity.
The number of foraging bees was assessed on 6 (out of 30) randomly selected strips of crop in each trial. Foraging activity was assessed 4 times per day at 10 am, 12 am, 2 pm and 4 pm on the days before the application. On the application day assessments were conducted at 10 am and 12 am before the application and 15 minutes, 1 hour and 3 hours after the application. After the application daily assessments were conducted at 10 am, 12 am, 2 pm and 4 pm. The behaviour of foraging honey bees was assessed.
The number of dead honey bees was determined on the alleys surrounding the 3 selected strips.

Findings and conclusion:
No changes in behaviour of the bees were observed, no increased mortality, neither at the hives, nor in the crop occurred. Repellent effects were not found. Thus, under the tested conditions, application of 25 g deltamethrin/ha did not cause adverse effects to bees.

*****

Supplemental information from the literature

The publications summarized below investigate residues of deltamethrin in bee-relevant matrices and are considered as supplemental information regarding the exposure profile of deltamethrin to bee-pollinators:

Report:	**KCP 10.3.1.6/11; Cossu, M.; Alamanni, M. C. (2003)**
Title:	Monitoring of pyrethroid residues in Sardinian honey by solid phase extraction and high-performance liquid chromatography.
Source:	Ital. J. Food Sci., Volume 15, Issue 4, Page 541-551, Publication Year 2003
DOI No: Document No:	- M-457696-01-1
Guidelines:	USP 23, The National Formulary 1995 – Validation of Compendial Methods: 1982, 12601 Twinbrook Parkway, Rockville, MD.
GLP:	-

EXECUTIVE SUMMARY
A rapid, economical and simplified multi-residue method, involving an extraction step and further HPLC analysis is described for the determination of fenpropathrin, cyfluthrin, deltamethrin and permethrin residues in honey. The method was tested on 74 honey samples, produced in Sardinia during 2000-2001. Pyrethroids were isolated from the matrix by means of solid- phase extraction (SPE) with octadecylsilane tandem-florisil cartridges. Recoveries of the pyrethroids at four different concentrations ranged from 93.6 to 99.2 %. The estimated limits of detection ranged from 0.2 to 1.6 µg/kg and limits of quantification ranged from 0.6 to 5.2 µg/kg. The stability of the four pyrethroids and their isomers was investigated on a multifloreal honey sample (spiked with 50 µg/kg). The

pesticides had a different degree of persistence in this matrix after 10 months. Deltamethrin had a loss of 40.1% 10 months after application. Traces of pyrethroid pesticides were detected in only four samples and their concentrations were lower than the limit of quantification. No residues were found in the other samples. Deltamethrin was not present in amounts above the limit of detection.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Chemical state and description:	-
Source of test item:	[redacted], Germany
Batch number:	-
Purity:	91-99%
Storage conditions:	-
Water solubility:	-

2. Site description

Location/country:	Sardinia (2000 – 2001)
Amount of samples:	74 honey samples
Source of the samples:	Beekeepers and local markets

B. Study design and methods

1. Test procedure

Test system (study type):	Monitoring study, Stability test
Duration of study	10 month (Stability test)
Application rate:	50 µg/kg (Stability test)
Test: unit	Closed glass containers
Sampling technique:	-
Sampling frequency:	Each month (Stability test)
Number of samples per site/soil type:	-
Sampling depth:	-
Transport/storage of samples:	5°C (Monitoring study; room temperature without direct exposure to sunlight (stability test)

2. Chemical analysis

Guideline/protocol:	
Method:	Solid-phase extraction and HPLC analysis
Extraction procedure:	Solid- phase extraction (SPE) with octadecylsilane tandem-florisil cartridges.
Analysis:	Hewlett-Packard 1050 series quaternary pump, variable-wavelength UV-Vis spectrophotometer detector and auto sampler with a 3390 integrator were used for quantification.
Reference item:	Equivalent to the test item
Recovery:	93.6-99.2% (deltamethrin: 97.3%)
Limit of detection:	0.1 – 1.6 µg/kg (deltamethrin: 0.2 µg/kg)
Limit of quantification:	0.6 - 5.2 µg/kg (deltamethrin 0.6 µg /kg)

RESULTS

1. Validity criteria:

The validation procedure, parameters and acceptance criteria were based on USP 23 (1995) guidelines and and recommendations in the literature. The method was validated regarding accuracy and precision.

2. Analytical findings:

Traces of pyrethroid pesticides were detected in only four samples and their concentrations were lower than the limit of quantification. No residues were found in the other samples. Deltamethrin was not present in amounts above the limit of detection. The stability test indicated that the degradation of deltamethrin was 40.1% 10 months after application

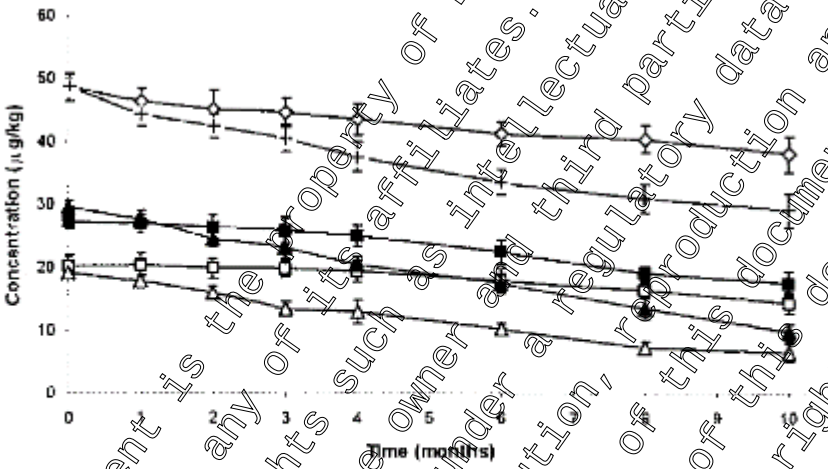


Fig. 5. Degradation of the four pyrethroids and their isomers in a honey sample spiked with 50 µg/kg of each insecticide evaluated (n=3) over a 10 month period: (○) fenpropathrin, (□) cyfluthrin 1, (■) cyfluthrin 2, (+) deltamethrin, (△) permethrin 1, (▲) permethrin 2.

RESULTS SUMMARY

Traces of pyrethroid pesticides were detected in only four samples and their concentrations were lower than the limit of quantification. No residues were found in the other samples. Deltamethrin was not present in amounts above the limit of detection. The stability test indicated that the degradation of deltamethrin was 40.1% 10 months after application

Comment of the Notifier: Cossu & Alamanni, M. C. (2003) in Italy investigated 74 honey samples obtained from beekeepers, local markets and supermarkets for the presence of deltamethrin and found no quantifiable deltamethrin residues.

Report:	**KCP 10.3.1.6/12; Campillo, N.; Penalver, R.; Aguinaga, N.; Hernandez-Cordoba, M. (2006)**
Title:	Solid-phase microextraction and gas chromatography with atomic emission detection for multiresidue determination of pesticides in honey.
Source:	Anal. Chim. Acta, 562, 1, p. 9-15
DOI No:	10.1016/j.aca.2006.01.034

Document No:	M-460886-01-1
Guidelines:	-
GLP:	-

EXECUTIVE SUMMARY

A method based on solid-phase microextraction (SPME) followed by gas chromatography with microwave-induced plasma atomic emission detection for determining 16 pesticides of different chemical families (organochlorines, organophosphorus compounds and pyrethrins) in honey is proposed. Parameters affecting the sample enrichment step, such as sample mass, ionic strength, absorption and desorption times and temperatures, were carefully optimized in the direct immersion mode. Element-specific detection and quantification was carried out by monitoring the chlorine (479 nm), bromine (478 nm) and sulphur (181 nm) emission lines, which provided nearly specific chromatograms. The matrix effect was evaluated for samples of different floral origin, it being concluded that standard addition calibration was required for quantification purposes. The detection limits ranged from 0.02 to 10 ng/g, depending on the compound and the honey sample under analysis. Six different honey samples were obtained from a local supermarket labelled as rosemary (samples 1–4), heather (sample 5) and orange blossom (sample 6). 1.5 g of honey samples were extracted using a SPME method and analysed using GC-AED. Each sample was performed in triplicates.
A recovery assay was conducted with spiked honey samples (4-3000 ng/ml). The spiked samples were set aside for 60 min at room temperature to let the methanol evaporate before sample analysis. The fortification procedure was applied to three different honey samples at four concentration levels and three replicates were analyzed in each case, corresponding to three aliquots of each sample independently fortified and analyzed.
None of the honeys analyzed contained the studied pesticides at least above the stated detection limits. An average recovery±S.D. (n = 64) of 91.4±15.4 was obtained.

MATERIAL AND METHODS

A. Material

1. Reference material

Reference item:	Deltamethrin
Active substance(s)	Deltamethrin
Chemical state and description:	
Source of test item:	[redacted], Germany)
Batch number:	-
Purity:	98-99.5%
Storage conditions:	4°C
Water solubility:	-

B. Study design and methods

1. Sampling

Test object::	Six different honey samples: osemary (samples 1–4), heather (sample 5) and orange blossom
Source of the test object:	Local supermarket
Number of replicates:	3 replicates
Storage of samples:	-

2. Chemical analysis

Guideline/protocol:	-

Method:	-
Pre-treatment of samples:	-
Conduction:	1.5 g of honey samples were extracted using a SPME method and analysed using GC-AED
Reference item:	Deltamethrin
Recovery:	91.4±15.4 (average recovery of all tested substances)
Limit of detection:	6.8 ng/g
Limit of quantification:	-

RESULTS

1. Validity criteria:

No validity criteria were stated.

2. Analytical findings:

None of the honeys analyzed contained the studied pesticides, at least above the stated detection limits. An average recovery±S.D. (n = 64) of 91.4±15.4 was obtained.

RESULTS SUMMARY

None of the honeys analyzed contained the studied pesticides, at least above the stated detection limits. An average recovery±S.D. (n = 64) of 91.4±15.4 was obtained.

Comment of the Notifier: Campillo *et al.* (2006) in Spain investigated six honey samples obtained from beekeepers, local markets and supermarkets for the presence of deltamethrin and found no quantifiable deltamethrin residues.

Report:	**KCP 10.3.1.6/13; Chauzat, M.-P.; Faucon, J.-P.; Martel, A.-C.; Lachaize, J.; Cougoule, N.; Aubert, M. (2006)**
Title:	A survey of pesticide residues in pollen loads collected by honey bees in France.
Source:	J. Econ. Entomol., Volume 99, Issue 2, Page 253-262, Publication Year 2006
DOI No:	http://dx.doi.org/10.1603/0022-0493-99.2.253
Document No:	M-455906-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

In 2002, a field survey was initiated on French apiaries to monitor weakness of honey bee, *Apis mellifera* L., colonies. Apiaries were evenly distributed in five sites located on continental France. Five colonies were randomly selected in each apiary, leading to a total of 125 studied honey bee colonies. For 3 year (starting in autumn 2002), colonies were visited four times per year: after winter, before summer, during summer and before winter. Pollen loads from traps were collected at each visit.

Multiresidue analyses were performed in pollen to search residues of 36 different molecules. Specific analyses were conducted to search fipronil and metabolites and also imidacloprid and metabolites. Residues of 19 searched compounds were found in samples. Contamination by pesticides ranged from 50 to 0%. Coumaphos and tau-fluvalinate residues were the most concentrated of all residues (mean concentrations were 925.0 and 487.2 g/kg, respectively). No deltamethrin residues were detected. Fipronil and metabolite contents were superior to the limit of detection in 16 samples. Residues of

fipronil were found in 10 samples. Nine samples contained the sulfone compound, and three samples contained the desulfinyl compound.
Residues of imidacloprid and 6-chloronicotinic acid were found in 69% of samples. Imidacloprid contents were quantified in 11 samples with values ranging from 1.1 to 5.7 g/kg. 6-Chloronicotinic acid content was superior to the limit of quantification in 28 samples with values ranging from 0.6 to 9.3 g/kg. Statistical tests showed no difference between places of sampling with the exception of fipronil. Possible origins of these contaminations, concentration and toxicity of pesticides, and the possible consequences for bees are discussed.

MATERIAL AND METHODS

A. Material

2. Site description

Location/country:	French
Amount of agricultural area:	5
History of site (crop, pesticides):	-
Temperature:	-
Precipitation:	-

B. Study design and methods

1. Sampling

Sampling technique:	Pollen traps
Sampling frequency:	four times a year
Number of samples per site:	2
Transport/storage of samples:	Coolbox/-20°C

4. Chemical analysis

Guideline/protocol:	-
Method:	Multiresidue analysis
Pre-treatment of samples:	Samples for the multiresidue analysis of pyrethroid insecticides were extracted with acetone and subsequently with dichloromethane after a liquid/liquid separation. A cleanup step with silica gel was performed. The two fractions were concentrated by evaporation. Residues obtained were dissolved in iso-octane for gas chromatographic analysis.
Conduction:	Multiresidue analysis was performed by gas chromatography by using an electron capture detector
Reference item:	-
Recovery:	-
Limit of detection:	0.1 to 57.0 µg/kg
Limit of quantification:	4.0 to 196.7 µg/kg

RESULTS

1. Validity criteria

No validity criteria were mentioned.

2. Analytical findings:

Multiresidue analyses were performed in pollen to search residues of 36 different molecules. Specific

analyses were conducted to search fipronil and metabolites and also imidacloprid and metabolites. Residues of 19 searched compounds were found in samples. Contamination by pesticides ranged from 50 to 0%. Coumaphos and tau-fluvalinate residues were the most concentrated of all residues (mean concentrations were 925.0 and 487.2 g/kg, respectively). No deltamethrin residues were detected. Fipronil and metabolite contents were superior to the limit of detection in 16 samples. Residues of fipronil were found in 10 samples. Nine samples contained the sulfone compound, and three samples contained the desulfinyl compound.
Residues of imidacloprid and 6-chloronicotinic acid were found in 69% of samples. Imidacloprid contents were quantified in 11 samples with values ranging from 1.1 to 5.7 g/kg. 6-Chloronicotinic acid content was superior to the limit of quantification in 28 samples with values ranging from 0.6 to 9.3 g/kg. Statistical tests showed no difference between places of sampling with the exception of fipronil.

Table 1: Pesticide residues in pole loads

	No. analysed samples	Number of positive samples	Frequency (%)	Residue concentrations		Average concentration (µg/kg)
				min. (µg/kg)	max. (µg/kg)	
Imidacloprid	81	40	49.4	>LOD	5.7	.12
6-Chloronicotinic acid	81	36	44.4	>LOD	9.3	1.2
Fipronil	81	10	12.4	>LOD	<LOQ	1.2
Fipronil desulfynil compound	81	9	11.1	>LOD	1.5	1.3
Penconazole	79	8	10.1	>LOD	126.0	27.6
Carbaryl	36	3	8.3	126.0	265.0	218.7
Endosulfan	82	5	6.1	>LOD	34.0	81.2
Tau-fluvalinate	82	5	6.1	>LOD	2020.0	487.2
Flusilazole	79	4	5.1	>LOD	71.0	26.1
Parathion-methyl	82	4	4.9	>LOD	<LOQ	24.8
Carbofuran	79	3	3.8	>LOD	10.9	14.0
Cyproconazole	79	3	3.8	>LOD	<LOQ	7.5
Fipronil sulfone compound	81	3	3.7	1.7	3.6	1.2
Myclobutanil	72	2	2.8	>LOD	20.3	13.9
Coumaphos	82	2	2.4	150.0	1700.0	925.0
Oxamyl	55	1	1.8	38.4	38.4	38.4
Tebuconazole	79	1	1.3	12.3	12.3	12.3
Hexaconazole	79	1	1.3	18.0	18.0	18.0
Parathion-ethyl	82	1	1.2	>LOD	<LOQ	19.2
Aldicarb	79	0	0.0	ND	ND	ND
Aldicarb sulfoxide	24	0	0.0	ND	ND	ND
Aldicarb sulfone	40	0	0.0	ND	ND	ND
Azinphos-methyl	82	0	0.0	ND	ND	ND
Chlorpyrifos-ethyl	82	0	0.0	ND	ND	ND
Cyfluthrin	82	0	0.0	ND	ND	ND
Cypermethrin	82	0	0.0	ND	ND	ND
Deltamethrin	82	0	0.0	ND	ND	ND
Dimetoate	82	0	0.0	ND	ND	ND
Epoxyconazole	79	0	0.0	ND	ND	ND
Fenitrothion	82	0	0.0	ND	ND	ND
Fenthion	82	0	0.0	ND	ND	ND
Lindane	82	0	0.0	ND	ND	ND
Malathion	82	0	0.0	ND	ND	ND
Mercaptodimethur	73	0	0.0	ND	ND	ND

	No. analysed samples	Number of positive samples	Frequency (%)	Residue concentrations		Average concentration (µg/kg)
				min. (µg/kg)	max. (µg/kg)	
Mercaptodimethur sulfone	71	0	0.0	ND	ND	ND
Mercaptodimethur sulfoxide	73	0	0.0	ND	ND	ND
Methidathion	82	0	0.0	ND	ND	ND
Methomyl	43	0	0.0	ND	ND	ND
Mevinphos	82	0	0.0	ND	ND	ND
Propiconazole	79	0	0.0	ND	ND	ND
Tetraconazole	79	0	0.0	ND	ND	ND

Pesticides are classified by decreasing frequencies (percentages). ND, not detected.

RESULTS SUMMARY

No deltamethrin residues were detected in pollen loads of five different apiaries in France.

Comment of the Notifier: Chauzat *et al.* (2006) investigated the residue situation of several plant protection products in pollen loads collected by honey bees in France. Apiaries were evenly distributed in five sites located on continental France. Five colonies were randomly selected in each apiary, leading to a total of 125 studied honey bee colonies. For 3 year (starting in autumn 2002), colonies were visited four times per year: after winter, before summer, during summer, and before winter. Pollen loads from traps were collected at each visit. In total, 82 pollen samples were subjected to deltamethrin residue analysis and in all samples no quantifiable deltamethrin residues were found.

Report:	**KCP 10.3.1.6/14; Chauzat, M.-P. ; Martel, A.-C. ; Cougoule, N.; Porta, P.; Lachaize, J.; Zeggane, S.; Aubert, M.; Faucon, J.-P. (2011)**
Title:	An assessment of honeybee colony matrices, Apis mellifera (Hymenoptera: Apidae) to monitor pesticide presence in continental France.
Source:	Environmental Toxicology and Chemistry, 30, 1, p. 103-111
DOI No:	10.1002/etc.361
Document No:	M-455993-01-1
Guidelines:	-
GLP:	-

EXECUTIVE SUMMARY

The aim of the present study was to assess the exposure of honeybees to pesticide residues [13] despite the fact that their active ingredients and commercialized substances were legal, regulated, and integral to food production. It provides an assessment of four different apicultural matrices to monitor pesticide presence in the environment over three years.

The studied apiaries were distributed among five sites in continental France covering the main zones of French honey production. Professional and hobbyist apiarists took part in the investigation. At the beginning of the study, 125 colonies (five honey bee colonies randomly selected in five apiaries from five different locations across France) were pesticide residues over 3 yr (2002-2005). The number of

[13] ChauzatMP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP. 2009. The influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environ Entomol 38:514–523.

surveyed hives was kept constant by replacing any dead colony by another one randomly selected from the same apiary. Colonies were visited four times per year: at the end of winter (March-April, visit A), before summer (may-June, visit B), during summer (July-August, visit C), and before winter (October-November, visit D). Adult bees were sampled at all visits, honey at visits B, C, and D, and beeswax only at visit D. Pollen traps were fixed on two extra colonies per apiary, and samples were taken at all visits when available. Individual colony samples, taken during the same visit, were pooled per apiary for analyses.

Forty-one different molecules were sought through individual (imidacloprid and fipronil) or multiresidue analyses. However, material and methods as well as results are summarized only for deltamethrin.

Insecticidal residues in honey were processed from 5 g of the honey samples that were homogenized with water and cleaned on Chem-Elut cartridges. After complete evaporation of the eluates, the extract was recovered with ethyl acetate for gas chromatography coupled with tandem mass chromatography (GC/MS/MS) analysis. For deltamethrin, the LOD and LOQ were 5 and 20 µg/kg respectively.

For multiresidue analysis, samples of pollen (10 g) were extracted using acetone extraction and liquid partitioning with dichloromethane. One clean-up was performed on a silica gel column for pesticides analysis. The two eluates were concentrated by complete evaporation under reduced pressure in a rotary evaporator using a 40°C water bath. Residues were dissolved in iso-octane for GC analysis. Multiresidue analysis was performed by GC using an electron-capture detector. The LOD for deltamethrin was 0.1 µg/kg and the LOQ was 39.9 µg/kg.

Beeswax samples (2 g) were extracted with n-hexane in an ultrasonic bath heated at 40°C. After freezing in liquid nitrogen and centrifugation, the supernatant fraction was collected and evaporated in a rotary evaporator (40°C) until 6 ml remained. Two liquid/liquid separations were performed with a mixture of n-hexane and acetonitrile. The acetonitrile phases were pooled together and concentrated on a rotary evaporator. The extract (2 ml) was cleaned on C18 cartridge. After elution with a mixture of acetonitrile and water, the solution was dried in a rotary evaporator and dissolved in 1 ml ethyl acetate for GC/MS/MS analysis. The LOD was 5 µg/kg, and the LOQ was 10 µg/kg.

Samples of honey bees (10 g) were extracted for multiresidue analysis using acetone extraction and liquid partitioning with dichloromethane. Clean-up with Florisil cartridge was performed for pesticides analysis. The two fractions obtained were concentrated by complete evaporation under reduced pressure in a rotary evaporator using a 40°C water bath. Residues were dissolved in iso-octane for the first eluate and in acetone for the second eluate. The two eluates were analyzed by GC analysis with specific detectors (ECD and NPD). The LOD and LOQ were 0.1 and 24.9 µg/kg, respectively.

The most frequent residue in pollen loads, honey, and honey bee matrices was imidacloprid or 6-chloronicotinic acid. Deltamethrin were found only in 5.9% of all analysed honey bee samples. In all other compartments (pollen loads, honey and beeswax), the proportion of positive samples (contaminated with deltamethrin) was lower. The average content of deltamethrin residues in positive samples collected in 120 French hives was between 39.0 µg/kg (Pollen), 14.7 µg/kg (Beeswax), 2.6 µg/kg (Honey) and 16.9 µg/kg (Honeybees).

MATERIAL AND METHODS

A. Material

1. Site description

Location/country:	France
Amount of sites:	5 sites in continental France
Amount of apiaries at test start:	5 apiaries
Amount of colonies at test start:	5 colonies
Cultivated crops:	Sunflower, canola, chestnut and local mixed flower honey

B. Study design and methods

1. Sampling

Samples:	Pollen loads, honey, honey bees and beeswax
Sampling frequency:	4 times (at the end of winter (March-April, visit A), before summer (May-June, visit B), during summer (July-August, visit C), and before winter (October-November, visit D)). Adult bees and Pollen loads were sampled at all visits, honey at visits B, C, and D, and beeswax only at visit D.
Transport/storage of samples:	Storage at -18°C

4. Chemical analysis

Guideline/protocol:	LOD and LOQ were calculated in accordance to the Guidance document on residue analytical methods [14]
Method:	Multiresidue analysis
Extraction (short description):	*Pollen loads:* 10 g pollen samples were extracted using acetone and liquid partitioning with dichloromethane. *Honey:* 5 g honey samples were homogenized with water and cleaned on Chem-Elut cartridges. After evaporation, the extract was recovered with ethyl acetate. *Honey bees:* Samples of honey bees (10 g) were extracted using acetone extraction and liquid partitioning with dichloromethane. *Beeswax:* Beeswax samples (2 g) were extracted with n-hexane in an ultrasonic bath.
Analysis apparatus:	Pollen loads: GC/ECD Honey: GC/MS/MS Honey bees: GC/ECD and GC/NPD Beeswax: GC/MS/MS
Reference item:	Certified pesticide standards were from CIL Cluzeau Info Labo (Sainte-Foy-La-Grance, France)
Recovery:	-
Limit of detection:	Pollen loads: 0.1 µg/kg Honey: 5 µg/kg Honey bees: 0.1 µg/kg Beeswax: 5 µg/kg
Limit of quantification:	Pollen loads: 29.9 µg/kg Honey: 20 µg/kg Honey bees: 24.9 µg/kg

[14] European Commission. 2007. Guidance on residue analytical methods. Document N° SANCO/825/00 rev. 6.

Beeswax: 10 µg/kg

RESULTS

1. Validity criteria:

The recoveries of analytes were calculated in each sequence of analyses and must be between 70 and 120%.

2. Analytical findings:

The most frequent residue in pollen loads, honey, and honey bee matrices was imidacloprid or 6-chloronicotinic acid. Deltamethrin were found only in 5.9% of all analysed honey bee samples. In all other compartments (pollen loads, honey and beeswax), the proportion of positive samples (contaminated with deltamethrin) was lower. The average content of deltamethrin residues in positive samples collected in 120 French hives was between 39.0 µg/kg (Pollen), 14.7 µg/kg (Beeswax), 2.6 µg/kg (Honey) and 16.9 µg/kg (Honeybees).

RESULTS SUMMARY

Deltamethrin were found only in 5.9% of all analysed honey bee samples. In all other compartments (pollen loads, honey and beeswax), the proportion of positive samples (contaminated with deltamethrin) was lower. The average content of deltamethrin residues in positive samples collected in 120 French hives was between 39.0 µg/kg (Pollen), 14.7 µg/kg (Beeswax), 2.6 µg/kg (Honey) and 16.9 µg/kg (Honeybees).

Comment of the Notifier: In 2011, Chauzat *et al.* investigated several hive matrices like honey, pollen and bee-wax. The studied apiaries were distributed among five sites in continental France covering the main zones of French honey production. Professional and hobbyist apiarists took part in the investigation. At the beginning of the study, 125 colonies (five honey bee colonies randomly selected in five apiaries from five different locations across France) were pesticide residues over 3 year (2002-2005). Out of 198 analysed pollen samples, quantifiable deltamethrin residues were found only in one single sample (0.5% of all samples investigated). Out of 237 analysed honey samples, collected from the hives under investigation, quantifiable deltamethrin residues were found only in two samples (0.8% of all samples investigated). Out of 87 analysed bee-wax samples, collected from the hives under investigation, quantifiable deltamethrin residues were found only in one single sample (1.1% of all samples investigated).

Report:	**KCP 10.3.1.6/15; Wiest, L.; Bulete, A.; Giroud, B.; Fratta, C.; Amic, S.; Lambert, O.; Pouliquen, H.; Arnaudguilhem, C. (2011)**
Title:	Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection.
Source:	J. Chromatogr., A,1218, 34, p. 5743-5756
DOI No:	-
Document No:	M-456064-01-1

Guidelines:	-
GLP:	-

EXECUTIVE SUMMARY

This paper presents an original analytical approach which consists in one simple extraction method for each matrix coupled with GC and LC analysis and a comprehensive validation of the whole method. Application to a large number of samples was made in order to check the robustness of the method but also to obtain a global view of environmental contaminants presence in beehives and to compare the contamination of honeys, honeybees and pollens. Material and methods as well as results are summarized for deltamethrin only.

The used multi residue analysis based on a modified "QuEChERS method", followed by gas chromatography coupled with Time of Flight mass spectrometry (GC-ToF). The "QuEChERS method" combines salting-out liquid-liquid extraction with acetonitrile and a dispersive-SPE clean up. It was adjusted to honey and especially to honeybee and pollen, by adding a small fraction of hexane in acetonitrile to eliminate lipids that interfere with mass spectrometry analysis. This method, combined with accurate and sensitive detection, allowed quantification and confirmation at levels as low as 10 ng/g, with recoveries between 60 and 120%. Application to more than 100 samples of each matrix was achieved. Samples were collected during the beekeeping season 2008 and 2009 (4 samplings per year: April/May, June/July, July/August, September/October). They concerned 16 apiaries of the "Région des pays de la Loire" (Western France) located in four types of landscapes (bocage, large-scale farming, gardening/orchards, urban area) and two control apiaries (less inhabited landscapes) located in Atlantic islands (Island of Yeu and Island of Ouessant).[15] For each period, samples were collected in several colonies of every apiary (honey, foraging bees and trap pollen) and repackaged to obtain one pool per apiary.

No deltamethrin were found in the tested samples. Deltamethrin recovery was 104-106, 81-98 and 69-88% for honeys, honeybees and pollens. The LOD and LOQ ranged between 4.6-28.9 and 16.2-57.8 ng/g, respectively

MATERIAL AND METHODS

A. Material

1. Sampling site

Location/country: "Région des Pays de la Loire" (Western France)
Amount of apiaries: 16
Type of landscape: Ocage, large-scale farming, gardening/ orchards, urban area
Pesticides used on fields: -
History of site (crop, pesticides): -

B. Study design and methods

1. Sampling

Samples: Honeybees, pollen and honey
Sampling frequency: 4 samplings per year (April/May, June/July, July/August,

[15] M. L'Hostis, H. Pouliquen, Annual report "L'Abeille mellifère (Apis mellifera) témoin de la pollution de l'environnement:étude sur un transect paysager en Pays de Loire" (2010).

	September/October)
Transport/storage of samples:	- 20°C
4. Chemical analysis	
Guideline/protocol:	-
Method:	Multi residue analysis
Pre-treatment of samples:	-
Conduction:	Multi residue analysis based on a modified "QuEChERS method", followed by gas chromatography coupled with Time of Flight mass spectrometry (GC-ToF). The "QuEChERS method" combines salting-out liquid-liquid extraction with acetonitrile and a dispersive-SPE clean up. It was adjusted to honey and especially to honeybee and pollen, by adding a small fraction of hexane in acetonitrile to eliminate lipids that interfere with mass spectrometry analysis
Reference item:	Deltamethrin (≥ 97%, Sigma-Aldrich, St. Quentin Fallavier, France)
Recovery:	Honey:104-106%; Honeybee: 81-98%; Pollen: 69-88%
Limit of detection:	Honeybee: 4.6 ng/g; honey:6.9 ng/g; pollen:28.9 ng/g
Limit of quantification:	Honeybee:16.2 ng/g; honey: 17.3 ng/g; pollen 57.8 ng/g

RESULTS

1. Validity criteria:

No validity criteria were stated.

2. Analytical findings:

No deltamethrin was found in honey, honeybee and pollen samples.

RESULTS SUMMARY

No deltamethrin was found in honey, honeybee and pollen samples.

Comment of the Notifier: Wiest *et al.* (2011) investigated bee-matrix samples collected in France during the beekeeping season 2008 and 2009. The samples were collected from16 apiaries of the "Région des pays de la Loire" (Western France) located in four types of landscapes (bocage, large-scale farming, gardening/ orchards, urban area) and two control apiaries (less inhabited landscapes) located in Atlantic islands (Island of Yeu and Island of Ouessant) . For each period, samples were collected in several colonies of every apiary (honey, foraging bees and trap pollen). No quantifiable deltamethrin residues were found.

Report:	**KCP 10.3.1.6/16; Lambert, O.; Piroux, M.; Puyo, S.; Thorin, C.; L'Hostis, M.; Wiest, L.; Buleté, A.; Delbac, F.; Pouliquen, H. (2013)**
Title:	Widespread Occurrence of Chemical Residues in Beehive Matrices from Apiaries Located in Different Landscapes of Western France
Source:	PLoS ONE (17 Jun 2013) 8(6): e67007
DOI No: Document No:	10.1371/journal.pone.0067007 M-465046-01-1
Guidelines:	None

GLP:	No. Published study (peer-reviewed article).

EXECUTIVE SUMMARY

Honey bee, honey and pollen samples collected from eighteen apiaries of western France from four different landscape contexts during four different periods in 2008 and in 2009 were analyzed to evaluate the presence of pesticides. A multi-residue analysis of 80 compounds was performed using a modified QuEChERS method, followed by GC-ToF and LC-MS/MS. A total of 37 different compounds were detected when considering all the matrices. Deltamethrin was not detected.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Deltamethrin
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	Not reported
Source of test item:	Not reported
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test area:

Location:	Western France (Bretagne and Pays de la Loire)
Field history:	18 apiaries located in four different landscapes: Two apiaries were located on small islands, selected to represent landscapes with low levels of pesticides. Six apiaries were located in a rural-grassland landscape characterized by high length of hedges and numerous grassland plots. Five apiaries were located in a rural-cultivated landscape characterized by large plots of crops (permanent, oil seed, grain crops, and market gardening) and a low hedgerow network. The pesticide display in these 11 rural-sites is reflective of agricultural practices and veterinary treatments of farm animals. Finally, five apiaries were located in an urban landscape characterized by large urban areas and some rural areas. The observed pesticides in these apiaries are reflective of leisure gardening.

3. Test organism(s)

Species:	Honey bee (*Apis mellifera* L.)
Source of test species:	18 apiaries located in four different landscapes in Western France (see above)
Age of test organisms at study initiation:	Not reported
Sample collection:	Three different biological matrices (foraging honey bees, trap pollen and honey) were collected from eight colonies randomly selected at each apiary. The honey bees were directly collected from the hive's flight board with a hand-held vacuum cleaner. The pollens were collected in pollen traps installed by beekeepers three days before the sampling. Honey samples were collected from

several honeycombs with a cutter or with a punch. Samples of honey bees, honey and pollen collected in the hives of the same apiary and at the same period were pooled. These field-collected pools were immediately placed on ice after sampling and then stored at -20°C until analysis.

B. Study design and methods

1. Test procedure

Test system (study type): Monitoring study

Duration of study: Over 2 years (2008 and 2009). The apiaries were visited four times each year: In spring, at the beginning of summer, in summer and at the beginning of autumn. During a single period, all samples were collected when possible within 10 days to minimize variations in climatic factors, flowering and pesticide treatments.

2. Chemical analysis

Guideline/protocol: No

Method: A multi-residue analysis of 80 compounds was performed using a modified ''QuEChERS method'' (''Quick Easy Cheap Effective Rugged Safe method''), followed by gas chromatography coupled with time-of-flight mass spectrometry (GC-ToF) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

Limit of detection: Honey bees: 4.6 ng deltamethrin/g; honey: 6.9 ng deltamethrin/g; pollen: 28.9 ng deltamethrin/g

Limit of quantification: Honey bees: 16.2 ng deltamethrin/g; honey: 17.3 ng deltamethrin/g; pollen: 57.8 ng deltamethrin/g

3. Statistical analysis:

Method: Linear mixed effects models were used to perform a comparison between the number of residues detected or quantified (i) in honey bees (n = 141), honey (n = 141) and pollen (n = 128); (ii) in different landscape structures (rural-grassland, rural-cultivated, urban and island); and (iii) for different sampling periods. Then, Tukey post-hoc tests (a specific version designed for mixed effects models) were used to implement multiple comparisons of the means in each model, (i) difference in matrix, (ii) difference in landscape and (iii) difference in sampling periods.

Software: The statistical analyses were performed using R software with the ''nlme package'' for the mixed effects and the ''multcomp package'' for the post-hoc tests.

RESULTS

1. Validity criteria:

No criteria.

2. Biological findings:

A total of 37 different compounds were detected when considering all the matrices. Deltamethrin was not detected.

RESULTS SUMMARY

A total of 37 different compounds were detected in honey bee, honey and pollen samples collected from eighteen apiaries of western France from four different landscape contexts during four different periods in 2008 and in 2009. Deltamethrin was not detected.

Comment of the Notifier: Lambert *et al*. (2013) most likely refers to the same dataset as Wiest *et al*. (2011), and reassured that no quantifiable deltamethrin residues were found in all investigated matrices.

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[16]).

Table 10.3.2- 1 Endpoints used for risk assessment

Test species, Dossier-File-No. Reference	Tested Formulation, Study Type, Exposure	Ecotoxicological Endpoint	
Typhlodromus pyri M-387027-01-1 Rep. no: B156TPL [redacted], 2010 KCA 8.3.2.2/01	Deltamethrin EW 16 Laboratory, glass plates 1.66 mg a.s./ha 3.32 mg a.s./ha 6.68 mg a.s./ha 13.44 mg a.s./ha 27.04 mg a.s./ha	LR_{50} 0.00439 g a.s./ha Corr. Mortality [%] 36 48 58 80 93	
Aphidius rhopalosiphi M-198587-01-1 Rep. no: AE014ARL Wientjes, 2000	Deltamethrin EW 15 Laboratory, glass plates 0.150 g a.s./ha 0.255 g a.s./ha 0.510 g a.s./ha 0.825 g a.s./ha 1.725 g a.s./ha 3.000 g a.s./ha	LR_{50} 1.726 g a.s./ha Corr. Mortality [%] 2 [A] 3 5 28 63 61	Effect on Reproduction [%] n.a. n.a. 39 79 n.a. n.a.
Typhlodromus pyri M-401577-01-1 Rep. no: CW10/086 [redacted], 2011 KCP 10.3.2.2/03	Deltamethrin EW 16 Extended lab., exposure on detached apple leaves 2.5 mg a.s./ha 5.5 mg a.s./ha 11.2 mg a.s./ha 23.6 mg a.s./ha 50.0 mg a.s./ha	LR_{50} 0.0165 g a.s./ha; ER_{50} >0.023.6 g a.s./ha Corr. Mortality [%] 12.4 21.3 47.2 49.4 80.9	Effect on Reproduction [%] 3.7 16.3 27.4 44.0 n.a.

[16] 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
Typhlodromus pyri M-419712-01-1 Rep.No: CW11/042 [redacted], 2011 KCP 10.3.2.2/04	Deltamethrin EW 15 Aged residues spray deposits on apple plants, 2 appl. of 12.5 g a.s./ha, 7 d interval. residues aged for 0 d: residues aged for 14 d: residues aged for 28 d: residues aged for 42 d: residues aged for 56 d:	Corr. Mortality [%] / Effect on Reproduction [%] 100.0 / n.a. 100.0 / n.a. 92.4 / n.a. 41.7 / 46.6 0.1 / 0.3
Aphidius rhopalosiphi M-400499-01-1 Rep. no: CW10/082 [redacted], 2011 KCP 10.3.2.2/05	Deltamethrin EW 15 Extended lab., exposure on potted barley plants 0.25 g a.s./ha 0.44 g a.s./ha 0.79 g a.s./ha 1.41 g a.s./ha 2.50 g a.s./ha	LR_{50} 1.79 g a.s./ha; ER_{50} >1.41 g a.s./ha Corr. Mortality [%] / Effect on Reproduction [%] / Repellency rel. to control [%] 3.3 / 31.1 / 7.5 n.sign. 3.3 / 21.0 / -7.5 [B] n.sign. 0.0 / 16.8 / 19.2 n.sign. 20.0 / 26.3 / 35.3 n.sign. 90.0 / n.a. / 24.4 n.sign.
Coccinella septempunctata M-401570-01-1 Rep. no: CW10/081 [redacted], 2011 KCP 10.3.2.2/06	Deltamethrin EW 15 Extended lab., exposure on detached apple leaves Control 8 mg a.s./ha 16 mg a.s./ha 32 mg a.s./ha 63 mg a.s./ha 125 mg a.s./ha	LR_{50} 0.02979 g a.s./ha Corr. Mortality [%] / Fertile Eggs/Female /Day / Hatching [%] - / 11.1 / 88.6 0.0 / 12.6 / 78.2 37.5 / 23.8 / 93.6 59.4 / 18.9 / 64.4 62.5 / n.a. / n.a. 90.6 / n.a. / n.a.
Chrysoperla carnea M-400889-01-1 Rep. no: CW10/085 [redacted], 2011 KCP 10.3.2.2/07	Deltamethrin EW 15 Extended lab., exposure on detached apple leaves Control 0.25 g a.s./ha 0.59 g a.s./ha 1.37 g a.s./ha 3.20 g a.s./ha 7.50 g a.s./ha	LR_{50} > 7.50 g a.s./ha Corr. Mortality [%] / Eggs/Female /Day / Hatching [%] - / 16.9 / 82.1 0.0 / 22.8 / 79.8 -5.4 [A] / 31.0 / 85.5 2.7 / 23.8 / 81.4 0.0 / 27.8 / 90.2 32.4 / 37.4 / 85.2
NTA off-crop field study (Netherlands) M-430876-03-1 Rep. no.: B158FFN [redacted], 2012 KCP 10.3.2.4/01	Deltamethrin EW 15 NTA full fauna off-crop field study. Spray application rates: 0.1, 0.23, 0.6, 1.3, 3 g a.s./ha	Community level NOER = 1.3 g a.s./ha Community level NOEAER = 3 g a.s./ha Population level NOER = 0.23 g a.s./ha Population level NOEAER = 3 g a.s./ha NOER: No Observed Effect Rate NOEAER: No Observed Ecologically Adverse Effect Rate
NTA off-crop field study (South-West France) M-430827-01-1 Rep. no.: B157FFN [redacted], 2012 KCP 10.3.2.4/02	Deltamethrin EW 15 NTA full fauna off-crop field study. Spray application rates: 0.1, 0.23, 0.6, 1.3, 3 g a.s./ha	Community level NOER = 3 g a.s./ha Population level NOER = 0.6 g a.s./ha Population level NOEAER = 3 g a.s./ha NOER: No Observed Effect Rate NOEAER: No Observed Ecologically Adverse Effect Rate

[A]: A negative value indicates a lower mortality in the treatment than in the control.
[B]: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.

RISK ASSESSMENT FOR OTHER NON-TARGET ARTHROPODS

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[17]).

The tier 1 non-target arthropod risk assessment is based on the LR_{50} values of the tier 1 laboratory studies conducted for *A. rhopalosiphi* and *T. pyri*.

Table 10.3.2- 2 Tier 1 HQ for terrestrial non-target arthropods for the in-field scenario

Crop	Species	Appl. rate [g a.s./ha]	MAF	LR_{50} [g a.s./ha]	HQ	Trigger	Refined risk assessment required
Sugarbeet	*T. pyri*	7.5	1	0.00439	1708	2	yes
	A. rhopalosiphi	7.5	1	1.726	4	2	yes
Cauliflower	*T. pyri*	7.5	1.7	0.00439	2904	2	yes
	A. rhopalosiphi	7.5	1.7	1.726	7	2	yes
Wheat	*T. pyri*	6.25	1.7	0.00439	2420	2	yes
	A. rhopalosiphi	6.25	1.7	1.726	6	2	yes

Table 10.3.2- 3 Tier 1 HQ for terrestrial non-target arthropods for the off-field scenario

Crop	Species	Appl. rate [g/ha]	MAF	Drift [%]	VDF	Correction factor	LR_{50} [g/ha]	HQ	Trig-ger
Sugar beet	*T. pyri*	7.5	1	2.77	10	10	0.21	47	2
	A. rhopalosiphi	7.5	1	2.77	10	10	0.21	0.12	2
Cauli-flower	*T. pyri*	7.5	1.7	2.38	10	10	0.30	69	2
	A. rhopalosiphi	7.5	1.7	2.38	10	10	0.30	0.18	2
Wheat	*T. pyri*	6.25	1.7	2.38	10	10	0.25	58	2
	A. rhopalosiphi	6.25	1.7	2.38	10	10	0.25	0.15	2

The tier 1 in-field and off-field HQ calculations indicate the need for a further refined risk assessment based on extended laboratory studies for *A. rhopalosiphi* and *T. pyri*. and two additional species.

Refined exposure assessment

The multiple application factor for the exposure assessment for deltamethrin can be refined based on DT_{50} values measured on leave material. [redacted] (M-192201-01-1) determined a DT_{50} of 2.8 days for residues of deltamethrin on foliage ([redacted], 1999; M-192201-01-1). This value was confirmed by [redacted] 2011, M-424226-01-1) which evaluated additional leave residue data from barley and spring barley and derived a DT_{50} of 2.9 days. Based on the DT_{50} values

[17] 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

of 2.8 to 2.9 days and the intended minimum application interval of 14 days for the uses in cauliflower and wheat the MAF can be refined to 1.1 (see Appendix III, ESCORT 2).

Refined in-field risk assessment

Table 10.3.2- 4 Tier 2 risk assessment for terrestrial non-target arthropods for the in-field scenario

Crop	Species	LR_{50}; ER_{50} [g/ha]	Appl. rate [g/ha]	MAF	In-field PEC_{max} [g/ha]	Effects are 50%?
Sugar beet	*T. pyri*	0.0165	7.5	1.0	7.5	No
	A. rhopalosiphi	1.41				No
	C. septempunctata	0.02979				No
	C. carnea	> 7.5				Yes
Cauliflower	*T. pyri*	0.0165	7.5	1.1	8.25	No
	A. rhopalosiphi	1.41				No
	C. septempunctata	0.02979				No
	C. carnea	7.5				No
Wheat	*T. pyri*	0.0165	6.25	1.1	6.88	No
	A. rhopalosiphi	1.41				No
	C. septempunctata	0.02979				No
	C. carnea	> 7.5				Yes

The tier 2 risk assessment indicates the need for a further refinement for the in-field area since initial effects on non-target arthropods like *A. rhopalosiphi*, *T. pyri*, and *C. septempunctata* are to be expected.

Refined in-field risk assessment

To demonstrate the potential for recovery in the in-field area an aged residue study has been conducted with *T. pyri* being the most sensitive species (M-419712-01-1). Deltamethrin EW 15 was applied two times with an spray interval of 7 days on apple leaves at a spray concentration of 12.5 g a.s./ha. After residues had aged for 42 days effects on mortality and reproduction were below 50%. After residues had aged for 56 days effects were 1% on mortality and 0.3% on reproduction. This indicates that the potential of recovery is given for non-target arthropods in the in-field scenario after two month, considering an application scheme of 2 applications, with a 7 day interval and a maximum application rate of 12.5 a.s./ha which even exceeds the intended use rates addressed in this dossier.

It can be concluded that no unacceptable adverse effects are to be expected on non-target arthropods in the in-field area from the use of Deltamethrin EW15 according to the proposed use pattern.

Refined off-field risk assessment

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

Crop	Species	LR_{50} [g/ha]	Appl. rate [g/ha]	MAF	Drift [%]	VDF	Correction factor	Off-field PEC_{max} [g/ha]	Effects are <50% ?
Sugar beet	*T. pyri*	0.01650	7.5	1.0	2.77	10	5	0.10	No
	A. rhopalosiphi	1.79				-	5	1.04	Yes
	C. septempunctata	0.02979				10	5	0.10	No
	C. carnea	> 7.50				10	5	0.10	Yes
Cauli-flower	*T. pyri*	0.01650	7.5	1.1	2.77	10	5	0.11	No
	A. rhopalosiphi	1.79				-	5	1.14	Yes
	C. septempunctata	0.02979				10	5	0.11	No
	C. carnea	> 7.50				10	5	0.11	Yes
Wheat	*T. pyri*	0.01650	6.25	1.1	2.77	10	5	0.10	No
	A. rhopalosiphi	1.79				-	5	0.95	Yes
	C. septempunctata	0.02979				10	5	0.10	No
	C. carnea	> 7.50				10	5	0.10	Yes

The refined off-field risk assessment has been conducted based on the results of the extended laboratory studies for *T. pyri*, *A. rhopalosiphi*, *C. septempunctata* and *C. carnea*. The risk assessment results for *T. pyri* and *C. septempunctata* indicate the need for a further refinement. This refined higher tier risk assessment will be based on the results of 2 full-fauna NTA off-crop field studies on grasslands.

Higher-tier off-field risk assessment

Two full-fauna NTA off-crop field studies on grasslands are available which were conducted in the Netherlands (M-430876-03-1, KCP 10.3.2.4/01) and in Southwestern France (M-430827-01-1, KCP 10.3.2.4/02) to assess off-field effects of Deltamethrin EW 15 under more realistic conditions. This study design has the advantage that an observed response would pertain to a representative, naturally occurring off-field NTA community.

Deltamethrin EW 15 was applied in a dose-response design at application rates of 0.1, 0.23, 0.6, 1.3 and 3.0 g a.s./ha to an uncultivated grassland in the Netherlands and in Southwestern France. Timing of the experiment (application in early July 2011) coincided with typical use patterns for the test item. Four replicate plots of 24 × 24 m were used per treatment (5 application rates, control, reference treatment = 28 plots in total). Arthropods were sampled comprehensively using three different sampling methods (pitfall, suction and weed/Berlese sampling) shortly before the application and 1, 2, 4-5 and 8 weeks after the application. Overall community changes relative to the control were analyzed using multivariate statistics and depicted by Principal Response Curves (PRC). In addition, effects on individual arthropod populations were analyzed with univariate statistics for taxa that were sufficiently abundant. The recommendations of the Dutch guidance document on the evaluation of NTA field studies (de Jong et al., 2010) were applied.
The toxic reference item treatments caused in both studies clear responses both at the arthropod community level and at the population level, demonstrating that the test systems were sufficiently sensitive to detect toxic effects.

Study results from the study in The Netherlands (M-430876-03-1, KCP 10.3.2.4/01):
Arthropod sampling showed that the study site held a diverse and representative insect and mite community, of which 62 taxa were sufficiently abundant for a univariate statistical evaluation at population level.
At the highest test rate of 3 g a.s./ha Deltamethrin EW 15 led to a statistically significant adverse effect on prevailing arthropod communities as evaluated by multivariate statistical analysis (PRC). This effect was limited to one sampling occasion one week after application in the suction- and the pitfall dataset. No statistically significant effects on arthropod communities were found at any of the rates tested up to and including 1.3 g a.s./ha. Therefore, 1.3 g a.s./ha is classified as the community NOER (No Observed Effect Rate) of Deltamethrin EW 15 and 3 g a.s./ha as the community NOEAER (No Observed Ecological Adverse Effect Rate).
The univariate statistical evaluation of the 62 taxa indicated that none of these taxa were adversely effected at 0.1 and 0.23 g a.s./ha. At 0.6 g a.s./ha, adult spiders of the genus *Pardosa* and gall midges of the family Cecidomyiidae showed adverse effects on only one sampling moment shortly after application. Three taxa (adult *Pardosa*, Cecidomyiidae and the chrysomelid beetles Alticinae) were adversely affected by treatment with Deltamethrin EW 15 applied at a rate of 1.3 g a.s./ha. These taxa all recovered within two to five weeks after application. At a rate of 3 g a.s./ha, nine taxa showed statistically significant adverse response patterns that were considered related to the test item treatment, with recovery occurring within two to eight weeks after application. Based on the observed recovery within the study period 3 g a.s./ha could be considered as the population NOEAER (No Observed Ecologically Adverse Effect Rate).

Study results from the study in Southwestern France (M-430827-01-1, KCP 10.3.2.4/02):
Also the study in Southwestern France covered a diverse and representative insect and mite community, of which 80 taxa were sufficiently abundant for a univariate statistical evaluation at population level. The evaluation at community level showed no effects up to and including the highest tested rate of 3 g a.s./ha (community NOER) as evaluated by multivariate statistical analysis (PRC).
At the population level, a dose related response of the taxon Thysanoptera could be observed in the two highest treatment groups (1.3 g and 3 g a.s./ha) one and two weeks after treatment which was statistically significant in the sampling immediately after application. Recovery occurred within two weeks. For the collembolan taxon Poduromorpha the starting densities were similar to the control. Populations developed similar in all test item treatments, but a statistically significant lower peak density was found in the highest test rate of 3 g a.s./ha two weeks after application. At the next sampling moment densities of this taxon was again similar to the control. In the highest test item rate (3 g a.s./ha) a decrease in numbers of adult Coccinellidae was found, up to two weeks after application. At the sampling 2 weeks after the treatment the difference to the control was statistically significant. Populations recovered till the next sampling and population dynamics were similar to the control during the remainder of the sampling period. Juvenile Cocinellini collected with suction did not show treatment related adverse effects.
Based on the evaluation of effects of Deltamethrin EW 15 at population level the application rate of 3 g a.s./ha can be classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate).

Conclusion

Since the higher tier risk assessment is based on 2 full fauna NTA studies that covered a wide range of species naturally occurring in off-field habitats, there is no need to add a correction factor in the exposure assessment to addresses uncertainty concerning the sensitivity of off-field arthropod species.

Table 10.3.2- 6 Higher tier assessment for terrestrial non-target arthropods for the off-field scenario

Crop	Appl. rate [g a.s./ha]	MAF	Drift [%]	**Off-field PEC_{max} [g a.s./ha]**	Species	**Community NOER [g/ha]**	Population NOEAER [g/ha]
Sugar beet	7.5	1	2.77%	**0.21**	off-crop field study NL	**1.3**	3.0
					off-crop field study F	**3.0**	3.0
Cauliflower	7.5	1.1	2.77%	**0.23**	off-crop field study NL	**1.3**	3.0
					off-crop field study F	**3.0**	3.0
Wheat	6.25	1.1	2.77%	**0.19**	off-crop field study NL	**1.3**	3.0
					off-crop field study F	**3.0**	3.0

The comparison of the off-field PEC values with the community NOER and the Populations NOEAER values for the 2 NTA off-crop field studies conducted in The Netherlands and in France indicate that no unacceptable adverse effects on non-target arthropods are to be expected from the use of Deltamethrin EW 15 according to the proposed use pattern.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

For the summaries of the tier 1 laboratory studies please refer to point KCA 8.3.2.2.

Supplemental information from the literature

Report:	**KCP 10.3.2.1/01; Saber, Moosa; Hejazi, Mir Jalil; Kamali, Karim; Moharramipour, Saeid (2005)**
Title:	Lethal and sublethal effects of fenitrothion and deltamethrin residues on the egg parasitoid Trissolcus grandis (Hymenoptera: Scelionidae).
Source:	J. Econ. Entomol. 98, 1, p. 35-40
DOI No:	http://dx.doi.org/10.1603/0022-0493-98.1.35
Document No:	M-460864-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The purpose of this study was to assess the total effects of fenitrothion and deltamethrin on immature and adult stages of *T. grandis*. However, material and methods as well as results are summarized for deltamethrin only.

The *T. grandis* colony used in all experiments originated from overwintering adults collected in October 1999 from a cherry orchard in Fashand-Karaj, Iran. The wasps were reared on *E. intergriceps* eggs for two generations in a growth chamber at 25 ± 1°C, 60 ± 10% RH, and a photoperiod of 16:8 (L:D) h. Adult wasps were provide with honey as a food source. The second generation (F2) of parasitoids was used in all experiments.

Preimaginal Development Bioassay. E. intergriceps eggs (< 24 h) were offered to young (< 24h) mated *T. grandis* females for 24 h at 25 ± 1°C, 60 ± 10% RH and a photoperiod of 16:8 (L:D) h. Parasitized eggs were exposed to 12.5 µg a.i./mL deltamethrin (Decis, 2.5 EC) at 2-, 4-, 6-, and 8-d-old preimaginal stages. Randomly taken parasitized egg masses were dipped in insecticide emulsion for 5 s. The control groups were submerged in distilled water only. Ten randomly chosen parasitized egg masses were exposed to the insecticides in each treatment, and the trial was repeated three times. After drying, each egg mass were then transferred to a small transparent plastic vial (15 by 100 mm). The vials were monitored daily for parasitoid emergence for 20 d. In the final assessment, the total number of eggs, parasitized eggs, and the emerged wasps were recorded.

Life table parameters: Adult *T. grandis* emerged from *E. integriceps* eggs treated at pupal stage (eighth day after parasitism) were used to measure the sublethal effects of the insecticides on the life table parameters of the surviving females. One hundred newly emerged females from three replicates of treated parasitized eggs were allowed to mate with sufficient number of males for 6 h. Then, 25 randomly chosen young female adults (<24 h old) were transferred individually to a Plexiglas cages used for holding adults (16 by 10 by 5 cm). Each female parasitoid was presented three *E. integriceps* fresh egg masses (42 eggs) and honey as food on a stripe of white paper. The egg masses were changed daily for each female until the female died. The parasitized eggs were stored at 25 ± 1°C, 60 ± 10% RH, and a photoperiod of 16:8 (L:D) h and allowed to emerge for 20 d. The total numbers of eggs, the numbers of black eggs (parasitized eggs) and emerged wasps, the sex of emerged wasps, and the number of eggs containing dead adults were recorded.

Lethal Concentration bioassay: Glass plates (14 by 15 cm) were sprayed with 1 mL of six concentrations ranging from 2.5 to 5.25 µg a.i./mL using a Potter Spray Tower. This resulted in homogeneous spray coverage of 0.92 ± 0.018 µL of fluid per square centimeter [92 L/ha]. Control plates were sprayed with distilled water. The plates were placed in laboratory for 1 h and allowed to dry completely. Then, 20 young female adults (<24 h) were placed in each exposure cage at 25 ± 1°C, 60 ± 10% RH, and a photoperiod of 16:8 [L:D] h. The number of dead and live wasps in each cage was counted 24 h after initial exposure to the insecticide residue. The treatment cages were monitored for at least 48 hafter recording the data, and very infrequent recoveries were taken into consideration and the data were adjusted. Each concentration consisted of two exposure cages and each experiment was replicated five times. Data were analysed using PROC PROBIT procedures to compute LC_{10}, LC_{50} and LC_{90} values on a standard and log scale with associated 95% fiducial limit.

The egg parasitoid *T. grandis* emergence from *E. integriceps* eggs were significantly affected by insecticides. Also, time of insecticide exposure relative to parasitoid preimaginal development significantly affected emergence. The lowest emergence rate occurred with E. integriceps parasitized eggs exposed to insecticides at the pupal stage (8 d after parasitism) in comparison with other stages. Deltamethrin reduced the emergence rate by 34.4%.

Examining effects of insecticides on life table parameters revealed that insecticides did not significantly affect mean longevity of *T. grandis*. Analysis of the reproductive activity of females that emerged from treated eggs at the pupal stage revealed no significant treatments effects on progeny production of females. And also the mean number of female offspring per female did not differ significantly compared with control. The proportion of males of the progeny was not significantly affected. Proportion of male offspring produced by *T. grandis* in the early life span of the parasitoid is higher in the treatments than in the control.

The LC_{50} was 3.9 µg a.i./mL after 24 h exposure plus at least 48 h monitoring.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis, 2.5 EC
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted] Germany
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Distilled water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Trissolcus grandis*
Cultivar:	-
Source of test species:	collected in October 1999 from a cherry orchard in Tashand-Karaj, Iran.
Age of test organisms at study initiation / Crop growth stage at treatment:	*Preimaginal development bioassay*: < 24 h *Life Table Parameters*: Adults from treated pupal stage *Lethal Concentration Bioassay:* < 24 h
Holding conditions prior to test:	25 ± 1°C, 60 ± 10% RH and a photoperiod of 16:8 (L:D) h
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Preimaginal development Bioassay, Life Table Parameters and Lethal Concentration Bioassay
Duration of study:	*Preimaginal development Bioassay*: 20 d; *Life Table Parameters*: until the female died plus the following 20 days for emergence observation; *Lethal Concentration Bioassay*: 24 h exposure plus 48 h monitoring
Treatments:	Deltamethrin and control (distilled water)
Test concentrations:	*Preimaginal development Bioassay*: 12.5 µg a.i./mL; *Life Table Parameters*:-; *Lethal Concentration Bioassay*: six concentrations ranging from 2.5 to 5.25 µg a.i./mL
Number of replicates:	*Preimaginal development Bioassay:* 3 replicates; *Life Table Parameters:* -; *Lethal Concentration Bioassay:* 5 replicates
Individuals per replicate:	*Preimaginal development Bioassay:* 10 ; *Life Table Parameters:* 25; *Lethal Concentration Bioassay:* 20
Test units (type and size):	*Preimaginal development Bioassay:* transparent plastic vial (15 by 100 mm); *Life Table Parameters:* Plexi glas cages (16 by 10 by 5 cm) ; *Lethal Concentration Bioassay:* Exposure cage with frame and two glass plates as the floor and ceiling; glass plates (14 by 15 cm)
Application / device / nozzles:	*Preimaginal development Bioassay:* egg masses were dipped in

	insecticide emulsion for 5 s; *Life Table Parameters:* -; *Lethal Concentration Bioassay:* Glass plates were sprayed with 1 mL at 14 mbar by using a Potter Spray Tower, 0.92 ± 0.018 µL/cm² [92 L/ha]
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	*Preimaginal development Bioassay:* direct contact; *Life Table Parameters:* -; *Lethal Concentration Bioassay:* Glass plates
Temperature / relative humidity:	25 ± 1°C, 60 ± 10% RH
Photoperiod:	16:8 (L:D) h
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	*Preimaginal development Bioassay:* total number of eggs, emerged wasps; *Life Table Parameters:* total number of eggs, number of black eggs, emerged wasps, sex of emerged wasp, number of eggs containing dead adults, longevity; *Lethal Concentration Bioassay:* number of dead and live wasps
Measurement frequency:	*Preimaginal development Bioassay:* daily; *Life Table Parameters:* daily; *Lethal Concentration Bioassay:* after 24 h plus following 48 h monitoring
Statistical analyses:	ANOVA, Tukey test, Fisher`s protected least significance difference (least significant difference, LSD); PROC GENMOD procedures and PROC PROBIT procedures

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

The egg parasitoid *T. grandis* emergence from *E. integriceps* eggs were significantly affected by insecticides. Also time of insecticide exposure relative to parasitoid preimaginal development significantly affected emergence. The lowest emergence rate occurred with E. integriceps parasitized eggs exposed to insecticides at the pupal stage (8 d after parasitism) in comparison with other stages. Deltamethrin reduced the emergence rate by 34.4%.

Examining effects of insecticides on life table parameters revealed that insecticides did not significantly affect mean longevity of *T. grandis*. Analysis of the reproductive activity of females that emerged from treated eggs at the pupal stage revealed no significant treatments effects on progeny production of females. And also the mean number of female offspring per female did not differ

significantly compared with control. The proportion of males of the progeny was not significantly affected. Proportion of male offspring produced by *T. grandis* in the early life span of the parasitoid is higher in the treatments than in the control.
The LC_{50} was 3.9 µg a.i./mL after 24 h exposure plus at least 48 h monitoring.

Table 1: Mean ± SE percentage emergence of *T. grandis* adults from *E. integriceps* eggs treated with deltamethrins at various days after *T. grandis* oviposition into eggs

Treatment	Field recommended concen (ppm) (g a.i./l)	No. days after oviposition				Mean % in treatments
		2	4	6	8	
Deltamethrin	500 (0.0125)	64.9 ± 5.7bB	64.7 ± 3.9cB	67.1 ± 6.0bB	61.9 ± 5.4bB	64.6 ± 2.6c
Control		98.6 ± 0.96aA	98.6 ± 0.96aA	98.6 ± 0.96aA	98.6 ± 0.96aA	98.6 ± 0.96a

Means in a column followed by different small letters or in a row by different capital letters are significantly different (Tukey test, α <.0.05).

Table 2: Mean ± SE sublethal effects of deltamethrin on life table parameters of *T. grandis*

Treatment	Longevity (d)	Progeny/female (M_x)	Female progeny/female (m_x)	Proportion males ♂/ ♀+♂
Deltamethrin	36.4 ± 3.7a	169.7 ± 9.8a	70.36 ± 15.1a	0.54 ± 0.1a
Control	38.6 ± 5.4a	221 ± 7.3a	105. ± 12.9a	0.37 ± 0.08a

Means within a column followed by different letters are significantly different (Fisher`s protected LSD, α < 0.05).

Table 3: Dose–response statistics for deltamethrin to adult *T. grandis*

N	Slope ± SE	Lethal concn (µg a.i./mL)		
		LC_{40} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
1200	4.15 ± 0.53	1.9 (1.5-2.2)	3.9 (3.6-4.2)	7.89 (6.9-10.4)

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 1996).

The LC50 was 3.9 µg a.i./mL after 24 h exposure plus at least 48 h monitoring.

Comment by the Notifier
Only the data from the lethal concentration bioassay allow the conversion into an application rate per area. The LC50 of 3.9 µg a.i./mL with an application volume of 92 L/ha is equivalent to 0.36 g a.i./ha. The presented data confirm the known acute toxicity of deltamethrin to arthropods under laboratory conditions. Compared to species like *T. pyri* or *C. septempunctata* this species shows a lower sensitivity. Therefore the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.1/02; Stefanello Junior, G. J.; Gruetzmacher, A. D.; Gruetzmacher, D. D.; Dalmazo, G. O.; Paschoal, M. D. F.; Haerter, W. R. (2008)**
Title:	The effect of insecticides used in corn crops on the parasitism capacity of Trichogramma pretiosum riley, 1879 (hymenoptera: trichogrammatidae). original title: efeito de inseticidas usados na cultura do milho sobre a capacidade de parasitismo de trichogramma pretiosum riley, 1879 (hymenoptera: trichogrammatidae).
Source:	Arqivos do Instituto Biologico Sao Paulo, 75, 2, pp. 187-194
DOI No:	-
Document No:	M-461229-01-2
Guidelines:	Hassan and Abdelgader (2001)[18], Hassan et al. (2000)[19]
GLP:	No

EXECUTIVE SUMMARY

The aim of this study is to assess the effect of registered insecticides for corn crops on the parasitism capacity of *Trichogramma pretiosum* under laboratory conditions using the methodology standardised by the "International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC), West Palaearctic Regional Section (WPRS)". Material and methods as well as results are summarized for deltamethrin only.

T. pretiosum were collected from a corn crop in the municipality of Pelotas, RS, and multiplied in the laboratory using eggs from the alternative host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae).

For the test of the parasitism capacity, deltamethrin (Decis 25 EC, 0.0025% a.i.) was diluted in distilled water to the maximum registered dosage for corn crops (field dosage: 0.20 l/ha), considering a spray solution volume of 200 l/ha. Then, the solution was sprayed on glass plates (13 cm x 13 cm, 0.2 cm thickness) which were later used to make the exposure cages according to Hassan ; Abdelgader (2001). A deposition film of solution of 1.75 ± 0.25 mg cm-2 was obtained by weighing the plates using a precision electronic balance.

Distilled water and Lorsban 480 BR were used as the negative and positive controls, respectively. Emergence tubes (glass vials 12 cm length x 2 cm diameter at one end x 0.7 cm at the opposite end), containing approximately 24-hour old adults of *T. pretiosum*, were connected to the exposure cages according to Hassan; Abdelgader (2001). Six hours after disconnecting the emergence tubes, cards containing three 1 cm circles with 450 ± 50 inviable eggs of *A. kuehniella* and food (solution composed of 200g of honey, 3 g of unflavoured jelly powder, and 100 ml of water) were provided at 24 (three cards), 48 (two cards) and 96 hours (one card) after spraying to be parasitised by *T. pretiosum*, totaling 18 circles, with approximately 9.000 eggs per cage. The evaluation of the parasitism capacity was carried out for up to 144 hours (6 days) and then the cages were taken apart and the cards were placed on Petri dishes (9.0 x 1.5 cm) which were stored under the same conditions as the test for three additional days so that the parasitised eggs would become dark enabling them to be

[18] Hassan, S.; Abdelgader, H. A sequential testing program to assess the side effects of pesticides on Trichogramma cacoeciae Marchal (Hym., Trichogrammatidae). Pesticides and Beneficial Organisms. IOBC/WPRS Bulletin, Darmstadt, v.24, n.4, p.71-81, 2001.

[19] Hassan, S.A.; Halsall, N.; Gray, A.P.; Kuehner, C.; Moll, M.; [redacted] M.; Roembke, J.; Yousef, A.; Nasr, F.; Abdelgader, H. A laboratory method to evaluate the side effects of plant protection products on Trichogramma cacoeciae Marchal (Hym., Trichogrammatidae), In: Candolfi, M.P.; Blumel, S.; Forster, R.; [redacted] M.; Grimm, C.; Hassan, S.A.; [redacted], U.; Mead-briggs, M.A.; Reber, B.; Schmuck, R.; Vogt, H. (Ed.). Guidelines to evaluate side-effects of plant protection products to non-target arthropods. Gent: IOBC/WPRS, 2000. p.107-119.

counted. Four replicates per treatment were used. The mean number of females per cage was 136.42. Test conditions were 25 ± 1 ºC, 70 ± 10% relative humidity and 14-hour photophase.
Deltamethrin caused 100% reduction in the parasitism capacity compared to the negative control. No eggs were parasitized per female. This effect was significant different compared to the control (Kurskal-Wallis, Bonferroni test) ($p > 0.05$).

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 25 EC [25 g a.s./L]
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Distilled water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Trichogramma pretiosum*
Cultivar:	-
Source of test species:	collected from a corn crop in the municipality of Pelotas, RS, and multiplied in the laboratory using eggs from the alternative host *Anagasta kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae)
Age of test organisms at study initiation / Crop growth stage at treatment:	24-hour old adults
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Dry residues of pesticides on glass plates
Treatments:	Deltamethrin, negative control (distilled water), positive control (Lorsban 480 BR)
Test concentrations:	0.20 L/ha [5 g a.s./ha]
Number of replicates:	4 replicates
Individuals per replicate:	Mean number: 136.42 of
Test conditions:	25 ± 1 ºC, 70 ± 10% relative humidity and 14-hour photophase
Test units (type and size):	glass plates (13 cm x 13 cm, 0.2 cm thickness) with exposure cages according to Hassan and Abdelgader (2001)[18]
Application / device / nozzles:	Sprayed with a deposit film of 1.75 ± 0.25 mg cm^{-2}
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Glass plates

Temperature / relative humidity:	25 ± 1 ºC, 70 ± 10% relative humidity
Photoperiod:	14-hour photophase
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Parasitism capacity
Measurement frequency:	Egg cards (450 ± 50 inviable eggs) were presented 24 (three cards), 48 (two cards) and 96 h (one card) after spraying; parasitism capacity was carried out for up to 144 hours (6 days)
Statistical analyses:	Kruskal-Wallis, Bonferroni test

RESULTS

Validity criteria:

No validity criteria were stated.

Biological findings:

Table 3: Effects of deltamethrin on the number of parasitised eggs per female, reduction (%) in the parasitism capacity of *Trichogramma pretiosum* and insecticide toxicity classification. Temperature 25 ± 1º C; relative humidity 70 ± 10%; photophase 14 hours, Pelotas, RS. 2006.

Commercial product/active ingredient	Females per cage	Eggs per female*		PR** (%)
Distilled water/negative control	141.14	35.07	a	-
Decis 25 EC/deltamethrin	136.42	0.00	c	100
Lorsban 480 BR/ **chlorpyrifos** ***	135.69	0.00	c	100

*Means followed by the same letters do not differ significantly by the (Kruskal-Wallis) Bonferroni test (p > 0.05); Test III (K = 22.76, p = 0.0004); Test IV (K=22.7629, p = 0.0004). The results express the mean of four replicates.

**PR = Reduction in the parasitism capacity of the treatments with insecticides compared to the negative control (distilled water).

***Positive control, insecticide recognised as being harmful by the IOBC/WPRS.

Deltamethrin at 5 g a.s./ha on glass plates caused 100% reduction in the parasitism capacity compared to the negative control (IOBC category 4: harmful). No eggs were parasitized per female. This effect was significant different compared to the control (Kurskal-Wallis, Bonferroni test) (p > 0.05).

Comment by the Notifier

The data on the parasitoid *Trichogramma pretiosum* a similar sensitivity compared to *A. rhopalosiphi* that has been tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	KCP 10.3.2.1/03; Angeli , G.; Baldessari, M; Maines, R.; Duso, C. (2005)
Title:	Side-effects of pesticides on the predatory bug *Orius laevigatus* (Heteroptera: Anthocoridae) in the laboratory
Source:	Biocontrol science and technology,15, 7 p. 745-754
DOI No:	
Document No:	M-460879-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

Laboratory trials were carried out in order to test the effects of 29 pesticides on the predatory bug, *Orius laevigatus*.
Residual exposure: To evaluate residual contact activity, newly moulted fourth instar nymphs of *O. laevigatus* were placed on treated Petri dishes and their mortality was checked after 7 days (2 predators per unit). The Decis SC (active ingredient: deltamethrin) concentration was 0.014 ml a.i./L applied with 1.7-1.8 mg/cm^2. Three replicates, each consisting of 20 predators in 10 Petri shres were used for each product, including a control treated with distilled water. The fecundity of surviving females was assessed every 3 days over a total of 14 days (three replicates with at least 5 females).
Exposure by ingestion: Ten, 2-3-day-old adults (sex ratio 1:1) collected from cultures were released in rearing units (180 x120 x 70 mm) for 12 days. Afterwards, treated eggs (0.014 ml a.i./L, Decis SC, applied with 1.7-1.8 mg/cm^2) were provided during the first and the third day of experiments; later, predators were supplied with untreated E. kuehniella eggs. Control groups were fed with E. kuehniella eggs treated with distilled water. Egg hatching was observed by considering at least 150 eggs per product. Three replicates were used for each pesticide including the control group. Adult mortality and female fecundity were recorded after 3, 5, 9 and 12 days from

In the residual exposure test unit, 100% of the fourth instar nymphs of *O. laevigatus* were assessed as dead. Consequently, no fecundity could be measured. Furthermore, toxicity of deltamethrin by ingestion to *O. laevigatus* was 100%.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Deltamethrin (Product: Decis SC)
Active substance(s): deltamethrin [active a.s. content in formulation not stated]
Adjuvant / Surfactant: -
Source of test item: -
Lot/Batch number: -
Purity: -
Storage conditions: -

2. Test solutions

Vehicle/solvent: Distilled water
Source of vehicle/solvent: -
Concentration of vehicle/solvent: -

3. Test organism(s)

Species: *Orius laevigatus*

Cultivar:	-
Source of test species:	Laboratory colonies of O. laevigatus were established in 1994 using a strain obtained from [redacted] (Italy), which originated from populations collected in Southern Italy
Age of test organisms at study initiation / Crop growth stage at treatment:	Residual exposure test: fourth instar nymphs; ingestion test: 2-3 days old adults
Holding conditions prior to test:	23±1°C, 75±10% RH, and photoperiod of 17L:7D h.
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Residual exposure test and ingestion test
Duration of study:	Residual exposure test: 21 days; ingestion test: 12 days
Treatments:	Residual exposure test: Pesticide solutions (8 mL) were applied to the lower surface of the two Petri dishes using a Potter Precision spray tower; ingestion test: The amount of pesticide solution deposited on the eggs, glued on thin pasteboard, was 1.7-2.8 mg/cm² (using the Potter Precision spray tower).
Test concentrations:	0.014 ml a.i./L equivalent to 60 mL/hL
Number of replicates:	3
Individuals per replicate:	Residual exposure test: 20 ind./10 petri dishes.; ingestion: 10 ind.
Test conditions:	23±1°C, 75±10% RH, and photoperiod of 17L:7D h.
Test units (type and size):	-
Application - device, nozzles:	Potter precision spray tower
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Surface of petri dishes and eggs of *E. kuehiniella*
Temperature / relative humidity:	-
Photoperiod:	-
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	-
Measurement frequency:	-
Statistical analyses:	One-way ANOVA and Tukey test (P < 0.01) (Mortality percentage values were transformed in arcsin square root)

RESULTS

Validity criteria:

Results were considered valid if control mortality did not exceed 15%. Observed control mortality was between 7.6 and 12%.

Biological findings:

Table 1: Toxicity of deltmaethrin after residual exposure and by ingestion to *O. laevigatus*

	Residual Exposure (days)		Ingestion	
	Mortality % (7 days)	Fecundity % (21 days)	Mortality % (12 days)	Fecundity % (12 days)
0.014 ml a.i./L	100	-	100	-

In the residual exposure test unit, 100% of the fourth instar nymphs of *O. laevigatus* were assessed as dead. Consequently, no fecundity could be measured. Furthermore, toxicity of deltamethrin by ingestion to *O. laevigatus* was 100%.

Comment by the Notifier

The tier 1 laboratory data and the oral exposure data for *Orius laevigatus* (100% mortality at 0.014 ml a.i./L with 1.7-1.8 mg/cm² equivalent to 2.4-2.5 g a.s./ha) indicate a sensitivity to deltamethrin seen also for other species tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.1/04; Talebi, K.; Karami, F.; Kowsari, A. A.; Bagheri, F. Editor(S): Jansen, J. P. (2010)**
Title:	Effects of three pesticides on predatory bug Orius albidipennis Reut. (Het.: Anthocoridae).
Source:	IOBC/WPRS Bulletin, 55, p. 19-22
DOI No:	
Document No:	M-482157-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

In this study we examined the effects of three pesticides on *Orius albidipennis* under laboratory conditions. Material and methods as well as results are summarized for deltamethrin only.

Pretadors collected from corn and alfalfa field in 2008 were reared in groups of 50 individuals in cylindrical plastic containers (8 x 18 cm). *Ephestia kuehniella* Zeller eggs and maize pollen were used as food. Rearing was conducted in chambers at a temperature of 27±1ºC, relative humidity of 65±5% and 16L:8D photoperiod.

Deltamethrin was tested at their recommended field rates (Decis 2.5 EC: 1.88 g a.i./ha; 12.5 µg a.i./ml). Glass plates (60×60mm) were treated with pesticides solutions using Potter precision spray tower (Burkard®) at 15 PSI pressure to get a deposit of 1.5mg/cm² of each pesticide solution. The control was treated with water. Drum-cells (50mm in diameter, 15mm high) were used for exposure of

individuals[20]. The top and the bottom of drum cells were closed with two treated glass plates. Ventilation was provided through eight holes (1cm diameter) covered with gauze, on the side of the cage. *E. kuehniella* eggs and maize pollen were placed in the cages. Ten individuals of 1st instar nymphs were introduced to each cell. The cells were maintained in an environmental chamber under the same conditions as rearing and mortality was recorded up to 7 days after treatment.
For deltamethrin, mortality was 37% and 67.29% (±17.48) after 24 h and seven days, respectively.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 2.5 EC
Active substance(s):	*Deltamethrin*
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Orius albidipennis*
Cultivar:	-
Source of test species:	corn and alfalfa field (2008)
Age of test organisms at study initiation /	1st instar nymph
Crop growth stage at treatment:	
Holding conditions prior to test:	27±1°C, relative humidity of 65±5% and 16L:8D photoperiod.
Acclimatisation:	

B. Study design and methods

1. Test procedure

Test system (study type):	Contact assay
Duration of study:	7 days
Treatments:	Deltamethrin and control (water)
Test concentrations:	1.88 g a.i./ha; 12.5 µg a.i./mL
Number of replicates:	not specified
Individuals per replicate:	10
Test conditions:	27±1°C, relative humidity of 65±5% and 16L:8D photoperiod.
Test units (type and size):	Drum-cells (50mm in diameter, 15mm high) with glass plates (60x60 mm) on the top and bottom
Application / device / nozzles:	Potter precision spray tower (Burkard®) (15 PSI; 1.5mg/cm²)
Water volume:	-
Calibration of sprayer:	-

[20] van de Veire, M., Smagghe, G. & D. Degheele, 1996: Laboratory test methods to evaluvate the effect of 31 pesticides on the predatory bug, Orius laevigatus. Entomogphaga. 41(2): 235-243.

2. Environmental conditions

Test medium:	Glass plates (60x60 mm)
Temperature / relative humidity:	27±1ºC, relative humidity of 65±5%
Photoperiod:	16L:8D photoperiod.
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	24 h and 7 days after treatment
Statistical analyses:	-

RESULTS

Validity criteria:

No validity criteria were stated.

Biological findings:

For deltamethrin, mortality was 37% and 67.29% (±17.48) after 24 h and seven days, respectively.

Comment by the Notifier

The tier 1 laboratory data on *Orius albidipennis* indicate a relative low sensitivity to deltamethrin in comparison to the some of the species tested for the regulatory data package (e.g. *T. pyri*, *C. septempunctata*). Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.1/05; Garcia, P.; Cabral, S.; Oliveira, L.; Rodrigues, A. (2006)**
Title:	Effects of deltamethrin on the reproduction of Trichogramma cordubensis (Hymenoptera: Trichogrammatidae).
Source:	Biocontrol science and technology, 16, 7-8, p. 699-708
DOI No:	10.1080/09583150600699911
Document No:	M-460896-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The influence of deltamethrin on the reproduction of *Trichogramma cordubensis*, a the lytokous egg parasitoid, was investigated by studying egg maturation and daily fecundity of insecticide treated wasps and offspring emergence rates.

Two concentrations of Decis 2.8% WG were tested: 12.5 and 23.6 mg a.i./L. All experiments were conducted at 22 ± 0.5 °C 70 ± 5% r.h. and 16L:8D. 15 groups of 10 females wasps (< 24 h old) were isolated in glass tubes. A drop of honey solution (10%) was offered as food and eggs of *E. kuehineiella* were presented to wasps on egg cards (400±10 eggs; < 24 h old). Treatments were applied 96 h after parasitism (prepupal stage) using a Potter`s Tower. Afterwards, egg cards were individually maintained until emergence of adult wasps. Then, the emerged wasps were used for two experiments:

Daily fecundity of treated wasps and offspring emergence rates. 30 emerged females per treatment were individually isolated in glass tubes containing a card with 100 ± 10 eggs of *E. kuehniella* with a drop of honey solution (10%). Egg cards were replaced every 24h with fresh ones during seven consecutive days to determine fecundity. Fecundity was determined by counting the number of parasitized host eggs that turned black. Emergence rates were estimated by dividing the number parasitized host eggs with emergence holes by the total number of parasitized host eggs.

Egg maturation. 15 emerged host-deprived females were dissected to determine the number of mature (> 71 µm length) eggs present in the four ovarioles of each wasp by using a light microscope. Furthermore, the number of mature eggs in the ovarioles of *T. cordubensis* was estimated after 24, 48, 72, 96, 120, 144 and 168 h of oviposition experience. Therefore, emerged females were individually isolated in glass tubes containing a card with 100 ± 10 eggs of *E. kuehniella* with a drop of honey solution (10%). Egg cards were replaced every day with fresh ones. Subsequent of every 24 h of parasitism, batches of 15 females from each treatment were dissected for observation of the mature eggs, following the above-mentioned procedure.

Results showed that the total number of parasitized eggs per female during 7 days was not significantly influenced by the tested concentrations of deltamethrin (one-way ANOVA). Wasps parasitized a significantly higher number of hosts on the first day of parasitism, sharply decreasing thereafter regardless the tested treatments (ANOVA Repeated Measures). Results indicated that for the higher concentration of deltamethrin (23.6 mg a.i./L) the emergence rates were high in the first 2 days (i.e. at 0 and 24 h), decreasing significantly on the 3rd and 4th day (i.e. at 48 and 72 h) (LSD tests). However, from the 5th day (i.e. at 96 h) onwards emergence rates increased, reaching to values similar to the control (LSD tests).

The egg maturation assessment indicated that the mean number of mature eggs per female decreased significantly after 24 h of oviposition. Throughout the following days, the number of mature eggs was relatively stable at low values, regardless of the treatments.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 2.8% EC
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	[redacted]
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Distilled water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Trichogramma cordubensis*
Cultivar:	-
Source of test species:	Parasitized eggs of *Autographa gamma* L. found at [redacted] (sao Miguel island, Azores). Laboratory reared ~12 generations on *Ephestia kuehniella*
Age of test organisms at study initiation / Crop growth stage at treatment:	Less than 24 h
Holding conditions prior to test:	20 ±1°C, 75 ±5% r.h. and L/D 16:D8
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	*Daily fecundity of treated wasps and offspring emergence rates* and *egg maturation*
Duration of study:	7 days
Treatments:	Control (distilled water) and Decis 2.8% EC diluted with distilled water
Test concentrations:	12.5 mg a.i./L and 23.6 mg a.i./L [952 L/ha => 11.9 g a.i./ha and 22.5 g a.i./ha]
Number of replicates:	-
Individuals per replicate:	*Daily fecundity of treated wasps and offspring emergence rates:* emerged 30 females per treatment; *egg maturation:* 15 females per treatment
Test units (type and size):	glass tubes (4 x 1 cm)
Application / device / nozzles:	Potter`s tower
Water volume:	9.52 ±2.17 µL/cm² [952 L/ha]
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Direct spray to egg cards
Temperature / relative humidity:	20 ±1°C / 75 ±5% r.h.
Photoperiod:	L/D 16:D8
Lighting:	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Daily fecundity of treated wasps, offspring emergence rates, number of mature eggs in the ovarioles
Measurement frequency:	Daily
Statistical analyses:	ANOVA, Fisher`s least significant difference test, ANOVA repeated mearusures procedure, Pearson`s correlation

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

Results showed that the total number of parasitized eggs per female during 7 days was not significantly influenced by the tested concentrations of deltamethrin (one-way ANOVA). Wasps parasitized a significantly higher number of hosts on the first day of parasitism, sharply decreasing thereafter regardless the tested treatments (ANOVA Repeated Measures). Results indicated that for the higher concentration of deltamethrin (23.6 mg a.i./L [22.5 g a.i./ha]) the emergence rates were high in the first 2 days (i.e. at 0 and 24 h), decreasing significantly on the 3rd and 4th day (i.e. at 48 and 72 h) (LSD tests). However, from the 5th day (i.e. at 96 h) onwards emergence rates increased, reaching to values similar to the control (LSD tests).

The egg maturation assessment indicated that the mean number of mature eggs per female decreased significantly after 24 h of oviposition. Throughout the following days, the number of mature eggs was relatively stable at low values, regardless of the treatments.

Comment by the Notifier

The data on the parasitoid *Trichogramma corduensis* treated during the prepupal stage indicated no relevant adverse effects by deltamethrin. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.1/06; Castilhos, R. V.; Gruetzmacher, A. D.; Nava, D. E.; Zotti, M. J.; Siqueira, P. R. B. (2011)**
Title:	Selectivity of pesticides used in peach orchard on adults of Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae). Seletividade de agrotoxicos utilizados em pomares de pessego a adultos do predador Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae).
Source:	Revista Brasileira de Fruticultura, 33, 1, p. 73-80
DOI No:	-
Document No:	M-462142-01-2
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The objective of this work was to evaluate the selectivity of pesticides used in integrated and conventional peach production on adults (males and females) of predator *C. externa,* in bioassays conducted in the laboratory. Material and methods as well as results are summarized for deltamethrin only.

Test concentration was 0.002 a.i. % (Decis 25 EC). Futhermore, a negative control (no pesticide) and a standard treatment of known toxicity, such as insecticide fenitrothion (Sumithion 500 EC) were performed.

The insects used in the bioassays originated from mass rearing in the laboratory (temperature of 25 ± 1

ºC, relative humidity of 70 ± 10% and 14 hours photophase). The bioassays consisted of exposure of adults of *C. externa* to dry residues of pesticides which had been sprayed on glass plates (12 x 12 cm) (floor and ceiling of cages), using a CO_2-pressurised sprayer equipped with a uniform flat spray tip (Teejet XR110015EVS) (pressure of 50 psi and spray solution film of 2 ± 0.2 mg.cm^{-2}). Each cage was made of a methacrylate ring (10 cm diameter x 3 cm thickness). The adults' diet, consisting of 15 ml of condensed milk, 2 egg yolks, 1 egg white, 30 g of honey, 20 g of sugar, 30 g of brewer's yeast, 50 g of wheat germ and 45 ml of distilled water, was supplied through the cage's side in sufficient quantity to carry out the bioassay. Afterwards, one week old predator adults, previously sorted by sex, were placed in the exposure cages. Each treatment consisted of four cages, each one containing five couples, each one being considered a replicate in the completely randomised design. The cumulative mortality of males and females, as well as the general mortality were evaluated at 24, 72 and 120 hours after exposure of the insects to the pesticides.
The values obtained for the number of dead insects were submitted to analysis of variance; the means were compared using the Tukey test, at 5% significance, using the WinStat statistics programme[21]
The mortality percentages were calculated for each treatment and were corrected relative to the control using the Schneider-Orelli formula[22].
Adult *C. externa* showed 24, 72, and 120 hours after the start of exposure to deltamethrin on glass plates 2.5%, 7.5%, and 32.5% mortality, respectively.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 25 EC
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Chrysoperla externa*
Cultivar:	-
Source of test species:	Laboratório do Núcleo de Manejo Integrado de Pragas (Centre for Integrated Pest Management Laboratory) (NUMIP) of the Embrapa Clima Temperado, at the laboratories of Controle Biológico e de Pesticidas (Biological and Pesticides Control), of the Universidade Federal de Pelotas, Capão do Leão - RS

[21] MACHADO, A.A.; CONCEIÇÃO, A.R. WinStat: sistema de análise estatística para windows. Universidade Federal de Pelotas, 2007. Disponível em <http://www.ufpel.edu.br/~machado>.
[22] PÜNTENER, W. Manual for field trials in plant protection. 2nd ed. Greensboro: Ciba- Geigy, Agricultural Division, 1981.

Age of test organisms at study initiation / Crop growth stage at treatment:	One week old adults
Holding conditions prior to test:	temperature of 25 ± 1 ºC, relative humidity of 70 ± 10% and 14 hours photophase
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Contact assay
Duration of study:	120 h
Treatments:	Decis 25 EC, negative control (no pesticide) and standard treatment (fenitrothion)
Test concentrations	0.002 a.i. %
Number of replicates:	4
Individuals per replicate:	5 couples
Test units (type and size):	Cages was made of a methacrylate ring (10 cm diameter x 3 cm thickness) with glass plates (12 x 12 cm) as floor and ceiling
Application / device / nozzles:	CO_2-pressurised sprayer (50 psi, 2 ± 0.2 mg cm^{-2})
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Glass plates
Temperature / relative humidity:	-
Photoperiod:	-
Lighting:	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	24, 72 and 120 hours after exposure
Statistical analyses:	Tukey test, at 5% significance, Schneider-Orelli formula

RESULTS

Validity criteria:

No validity criteria were stated.

Biological findings:

Table: Cumulative mortality (No. ± SE) for females and males at 24, 72 and 120 after the start of exposure of the adult phase of *Chrysoperla externa* for Decis 25 EC

Treatment	D.C.*	M[1] [24 hours]		M[2] [72 hours]		M[3] [120 hours]	
		♀	♂	♀	♂	♀	♂
Control	-	0.0 ± 0.0 bA	0.0 ± 0.0 bA	0.0 ± 0.0 bA	0.0 ± 0.0 bA	0.0 ± 0.0 bA	0.0 ± 0.0 bA
Deltameth	40	0.0 ± 0.0 bA	0.3 ± 0.5 bA	0.0 ± 0.0 bA	0.8 ± 1.0 bA	0.5 ± 0.6 bB	2.8 ± 1.0 bA
Fenitrothio	155	3.8 ± 1.0 aB	5.0 ± 0.0 aA	5.0 ± 0.0 aA	5.0 ± 0.0 aA	0.5 ± 0.6 bB	5.0 ± 0.0 aA

*D.C. = Dosage of commercial formulation (g or ml• 100 l-1) •θ l• ha-1; 1Mean values obtained from four replicates with five couples in each. Means followed by same letter, lowercase in column and uppercase in rows, for each evaluation time, are not significantly different from each other by the Tukey test at 5% probability.

Table: Cumulative mortality (No. ± SE) at 24, 72 and 120 after the start of exposure of the adults of Chrysoperla externa to residues of registered pesticides used in peach crops.

Treatment	D.C.*	M [24 hours]		M [72 hours]		M [120 hours]	
		No. ± SE[1]	%**	No. ± SE[1]	%**	No. ± SE[1]	%**
Control	-	0.0 ± 0.0 b	0.0	0.0 ± 0.0 b	0.0	0.0 ± 0.0 c	0.0
Deltamethrin	40	0.3 ± 0.5 b	2.5	0.8 ± 1.0 b	7.5	3.3 ± 1.0 b	32.5
Fenitrothion	150	8.8 ± 1.0 a	82.5	10.0 ± 0.0 a	100.0	10.0 ± 0.0 a	100.0

*D.C. = Dosage of commercial formulation (g or ml• 100 l-1) •θ l• ha-1; **Corrected mortality by the Schneider-Orelli formula

Adult *C. externa* showed 24, 72 and 120 hours after the start of exposure to deltamethrin on glass plates 2.5%, 7.5%, and 32.5% mortality, respectively.

Comment by the Notifier

The publication confirms as known from the regulatory non-target arthropod data package that *Chrysoperla* species show compared to other tested non-target arthropods species like *T. pyri* or *C. septempunctata* a lower sensitivity concerning exposure to deltamethrin. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.1/07; Torres, F. Z. V.; Carvalho, G. A.; Rodrigues De Sousza., J.; Rocha, L. C. D. (2007)**
Title:	Toxicity evaluation of insecticides used in rose crops to adults of Orius insidiosus (Say) (Hemiptera: Anthocoridae)
Source:	Acta Sci., Agron., 29, 3, pp. 323-329
DOI No:	
Document No:	M-460911-01-2
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The objective of this work was to evaluate the toxicity of some insecticides including deltamethrin, used in rose crops, to adults of *O. insidiosus*. Material and methods as well as results are summarized for deltamethrin only.

As test substance was used deltamethrin (0.0008 g a.i./100 mL) (Decis® 25 CE). The control treatment was distilled water only.

Forty adults aged up to 48 hours were used in each treatment; they were obtained from a population bred in the laboratory and were placed in 15 cm diameter Petri dishes. Then, they were sprayed in a

Potter tower calibrated to 15 lb in^{-2}, ensuring the application of 1.5 ± 0.5 mg cm^{-2} spray solution. After being sprayed, the insects were placed one in each 5 cm diameter Petri dish containing a lump of cotton wool moistened with distilled water to keep the interior of the dish moist. The food source was non-viable eggs of A. *kuehniella* provided ad libitum to the insects. The dishes were sealed and kept in a climatic chamber set at 25 ± 2ºC, RH 70 ± 10% and 12-hour photophase. Food was supplied every 48 hours and the cotton wool was moistened every 24 hours. Four replicates were performed with ten insects in each.
Mortality of the adults was evaluated at 1, 3, 6, 12, 24 hours after the application of the products, with a stereomicroscope (40 x), where the adults not moving to the touch of a paintbrush were considered dead.
The data for the mortality in adults were subjected to analysis of variance to compare the products amongst themselves and for the evaluation of the effects of the products with time, the fit to a regression equation was tested. Furthermore, whenever the ANOVA F-test was significant, comparison of the means was carried out using the Scott-Knott test with 5% significance[23]
Deltamethrin caused 3% mortality 3 hours after the spraying and at 24 h, all treated *O. insidiosus* adults were assessed as dead (mortality 100%).

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis® 25 CE
Active substance(s):	Deltamethrin
Adjuvant/Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Orius insidiosus*
Cultivar:	-
Source of test species:	Population bred in the laboratory
Age of test organisms at study initiation / Crop growth stage at treatment:	Up to 48 hours
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Mortality bioassay (contact)
Duration of study:	120 hours
Treatments:	Deltamethrin, control (distilled water)

[23] SCOTT, A.J.; KNOTT, M.A. A cluster analysis method for grouping means in the analysis of variance. Biometrics, Washington, D.C., v. 30, p. 507-512, 1974.

Test concentrations	0.0008 g a.i./ 100 mL
Number of replicates:	4 replicates
Individuals per replicate:	10 insects
Test conditions:	25 ± 2ºC, RH 70 ± 10% and 12-hour photophase
Test units (type and size):	5 cm diameter petri dish
Application / device / nozzles:	Potter tower (15 lb in^{-2}, 1.5 ± 0.5 mg/cm^{-2})
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Direct spraying
Temperature / relative humidity:	25 ± 2ºC, RH 70 ± 10%
Photoperiod:	12-hour photophase
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	1, 3, 6, 12, 24 hours
Statistical analyses:	F-test, Scott-Knott test[23]

RESULTS

Validity criteria:

No validity criteria were stated.

Biological findings:

Deltamethrin caused 3% mortality 3 hours after the spraying and at 24 h, all treated *O. insidiosus* adults were assessed as dead (mortality: 100%).

Comment by the Notifier

The tier 1 laboratory data for *Orius insidiosus* (100% mortality after 24 h at 0.0008 g a.i./ 100 mL with 1.5 mg/cm²; equivalent to 1.2 g a.s./ha) indicate a sensitivity to deltamethrin seen also for other species tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.1/08; Godoy, M. S.; Carvalho, G. A.; Moraes, J. C.; Junior, M. G.; Morais, A. A.; Cosme, L. V. (2004)**
Title:	Selectivity of insecticides used in citrus crops to eggs and larvae of Chrysoperla externa (Hagen) (Neuroptera: Chrysopidae). Seletividade de inseticidas utilizados na cultura dos citros para ovos e larvas de Chrysoperla externa (Hagen) (Neuroptera: Chrysopidae).

Source:	Neotropical Entomology, 33, 5, p. 639-646
DOI No: Document No:	- M-460874-01-2
Guidelines:	Hassan et al. 1991[24]; IOBC/WPRS 1992[25]; Hassan and Degrande 1996[26]
GLP:	No

EXECUTIVE SUMMARY

The purpose of this work was to evaluate the effects of some pesticides used in citrus groves on the eggs and larvae of *Chrysoperla externa*. Material and methods as well as results are summarized for deltamethrin only.

Test concentration was 0.0125 g a.i./L of Decis 25 CE. The control treatment comprised of water only. The sprays were applied by means of a Potter tower at a pressure of 15 psi with an application volume of 1.5 ± 0.5 mg/cm^2.

Effects on eggs: For each treatment, thirty *C. externa* eggs up to twelve hours old were placed in 15-cm diameter Petri dishes and sprayed with deltamethrin or distilled water. After spraying, the dishes were kept in the dark for two hours to reduce the moisture level on the egg surface, after which they were individually placed in glass tubes of 2.5 cm diameter and 8 cm length and kept in a climatised room at 25 ± 2°C, r.h. 70 ± 10% and 12-hour photophase.

The eggs were evaluated daily until emergence of the larvae. The latter were fed ad libitum with eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) until they changed into pupae. These latter were kept in the glass tubes until the adults emerged. The adults were grouped into pairs and distributed as one pair in each PVC container of 15 cm diameter and 10 cm height, with a minimum of five and a maximum of 15 pairs per treatment. The adults were kept under the same ambient conditions as described above and were fed with brewer's yeast and honey (1:1, v/v).

The numbers of eggs laid were evaluated every three days for four consecutive weeks. In addition, 100 eggs per treatment were collected and placed individually in the wells of ELISA microtitration plates covered with PVC film and kept in a climatised room.

The experimental design used was entirely randomised with the seven aforesaid treatments and ten repetitions, with each parcel comprising three eggs. The viability of the eggs and survival of the larvae, pupae and adults was evaluated together with the daily and total oviposition per female.

Effect on larvae: The test unit comprised a glass plate of 11.6 cm length, 9.6 cm width, which was sprayed with the insecticides by means of a Potter tower with the setting described above. After spraying, the plates were places in the climatised room.

Larvae of the first, second and third instars up to 24 hours old, obtained from the laboratory vivarium, were individually placed on the glass plates and fed ad libitum with *A. kuehniella* eggs until they changed into pupae, when they were transferred to glass tubes of 2.5 cm diameter and 8 cm height closed on top with PVC film and kept in the climatised room. The adults obtained were maintained

[24] Hassan, S.A., F. Bigler, H. Bogenshuetz, E. Boller, J. Brun, J.N.M. Calis, P. Chiverton, J. Coresmans-Pelseneer, C. Duso, G.B. Lewis, E. Mansour, L. Moreth, P.A. Oomen, L. Polgar, W. Rieckmann, L. Samsøe-Petersen, A. Staubli, G. Sterk, K. Tavares, J.J. Tuset & G. Viggiani. 1991. Results of the fifth joint pesticide testing programme carried out by the IOBC/WPRS – Working Group "Pesticides and Beneficial Organisms". Entomophaga 36: 55-67.

[25] International Organization for Biological Control. West Palaearctic Regional Section. 1992. Working Group "Pesticides and Beneficial Organisms", Guidelines for testing the effects of pesticides on beneficial organisms: description of test methods. Bulletin IOBC/WPRS 15: 1- 186.

[26] Hassan, S.A. & P.E. Degrande. 1996. Methods to test the side effects of pesticides on Trichogramma, p.63-74. In J.R.P. Parra & R.A. Zucchi (eds.), Trichogramma e o controle biológico aplicado. Piracicaba, FEALQ, 324p.

and fed in the same way as described in the bioassay with eggs. The number of eggs in each container was recorded every three days for four consecutive weeks and viability was determined as described in the previous bioassay with adults arising from eggs treated with the insecticides.
The experimental design used was entirely randomised with the seven aforesaid treatments and ten repetitions, with each parcel comprising three first, second or third instar larvae. The control treatment comprised distilled water only. The survival of the larvae and pupa, the daily and total oviposition per female and the viability of the eggs were evaluated.
The data for daily and total oviposition, egg viability and sex ratio underwent analysis of variance and the means were compared by the Scott-Knott grouping test at 5% significance[27]. Mortality data were corrected by the Abbott formula (1925)[28] before undergoing analysis of variance.
When the insecticides were sprayed onto *C. externa* eggs, there were no significant differences between the evaluated treatments, with viability of 83.3% for control treatment and 76.6%.
Deltamethrin significantly reduced the survival of first instar larvae compared to the other treatments, with a mean of 38.3% compared 95.0% in the control treatment. The insecticide did not affect the survival of second and third instars or the pupae of *C. externa*.
For *C. externa* larvae treated in the first, second and third instar, significant harmful effects were found with survival rates of 0%.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Decis 25 CE
Active substance(s): Deltamethrin
Adjuvant / Surfactant: -
Source of test item: -
Lot/Batch number: -
Purity: -
Storage conditions: -

2. Test solutions

Vehicle/solvent: Water
Source of vehicle/solvent: -
Concentration of vehicle/solvent: -

3. Test organism(s)

Species: *Chrysoperla externa*
Cultivar: -
Source of test species: -
Age of test organisms at study initiation: Larvae, first instar, second instar and third instar
Crop growth stage at treatment:
Holding conditions prior to test: 25 ± 2°C, r.h. 70 ± 10% and 12-hour photophase.
Acclimatisation: -

B. Study design and methods

1. Test procedure

Test system (study type): Direct spray and contact assay

[27] Scott, A.J. & M.A. Knott. 1974. A cluster analyses method for grouping means in the analyses of variance. Biometrics 30: 502-512.
[28] Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Duration of study:	Ca. 4 weeks
Treatments:	Deltamethrin and Control (water)
Test concentrations	0.0125 g a.i./L
Number of replicates:	10
Individuals per replicate:	30 eggs per treatment (each parcel with 3 eggs); For first, second and third instar: each parcel comprised three larvae
Test units (type and size):	*Effect on eggs:* Egg: glass tubes of 2.5 cm diameter and 8 cm length; Adults: PVC container of 15 cm diameter and 10 cm height; *Effect on larvae:* larvae: glass plate 11.6 cm length, 9.6 cm width; pupae: 2.5 cm diameter and 8 cm height
Application / device / nozzles:	Potter tower (15 psi of 1.5 ± 0.5 mg/cm²)
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Eggs: direct spraying on eggs; larvae: treated glass plates
Temperature / relative humidity:	at 25 ± 2°C, r.h. 70 ± 10%
Photoperiod:	12-hour photophase
Lighting	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Viability, number of eggs
Measurement frequency:	Egg viability: daily, number of eggs laid: every three days;
Statistical analyses:	ANOVA, Scott-Knott grouping test[27] at 5% significance Abbott formula (1925)[28]

RESULTS

Validity criteria:

No validity criteria were stated.

Biological findings:

Table 1: Viability (%) of eggs and survival (%) of first, second and third instar larvae and pupa (± SE) of

***C. externa* derived from eggs sprayed with Decis 25 CE**

Treatment	Egg	Instar			Pupa
		First	Second	Third	
Deltamethrin	76.6 ± 7.14 a	38.3±10.31 b	100 ± 0.07 a	100 ± 0.08 a	100 ± 0.08 a
Control	83.3 ± 7.51 a	95.0 ± 5.06 a	96.7 ± 3.32 a	100 ± 0.08 a	100 ± 0.09 a

Means followed by the same letter in a column do not differ significantly from each other by the Scott-Knott test (P ≤ 0.05).

Table 2: Percentage mortality of C. extern and mean number of eggs/day/female when deltamethrin was sprayed onto Chrysopidae eggs

Treatments	M	R1	R2	E	IOBC Class
Deltamethrin	73.3	10.0	89.6	74.4	2 (slightly harmful)
Control	23.2	12.8	95.4	-	-
Treatment	Egg	Instar			

M = Total mortality (%) of C. externa.
R1 = Number of eggs/day/female.
R2 = Viability (%) of eggs collected in the period

Table 3: Survival (%) (± SE) of first, second and third instar larvae and pupa of *C. externa* derived from first, second and third instar larvae exposed to contact treatment with deltamethrin.

Treatment	Instar			Pupa
	First	Second	Third	
Deltamethrin	0.0 ± 0.08 d	-	-	-
Control	83.3 ± 5.52 a	100 ± 0.09	91.7 ± 5.71	96.7 ± 3.34

Means followed by the same letter in a column do not differ significantly from each other by the Scott-Knott test (P ≤ 0.05).

When the insecticides were sprayed onto *C. externa* eggs, there were no significant differences between the evaluated treatments, with viability of 83.3% for control treatment and 76.6% for the deltamethrin treatment. Deltamethrin significantly reduced the survival of first instar larvae compared to the other treatments, with a mean of 38.3%. The insecticide did not affect the survival of second and third instars or the pupae of *C. externa*.
For *C. externa* larvae treated in the first, second or third instar, significant harmful effects were found with survival rates of 0%.

Comment by the Notifier
The tier 1 laboratory data for *Chrysoperla externa* (100% mortality of exposed larvae at 1.9 g a.s./ha) indicate a sensitivity to deltamethrin seen also for other species tested in tier 1 test systems for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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

Report:	**KCP 10.3.2.2/01, [redacted] (2011)**
Title:	Toxicity to the predatory mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) using an extended laboratory test on *Malus sylvestris* Deltamethrin EW 15 g/L
Document No.:	M-401577-01-1 (Rep. No.: CW10/086)
Guidelines:	Blümel et al. (2000), Candolfi et al. (2001)
GLP	GLP study

Materials and methods

The oil in water emulsion formulation of Deltamethrin EW 15 g/L was tested, specified by sample description: TOX 08992-00; specification no.: 102000013165-05; batch ID: 2010-002975 [analysed content of active ingredient: Deltamethrin 15.35 g/L]; density: 1.023 g/mL.
The test item was applied onto detached leaves of *Malus sylvestris* at rates of 2.5, 5.5, 11.2, 23.6 and 50.0 mg as/ha and the effects on the predatory mite *Typhlodromus pyri* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 15 g as/ha was included to indicate the relative susceptibility of the test organisms and the test system.
Mortality of 100 mites (10 replicates with 10 individuals per test group) was assessed 7, 10, 12 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.
Due to the known repellent effects of the test item the mortality part of this study was performed in closed, actively ventilated cells (Munger cages). On day 7 after application the surviving mites were transferred on untreated open exposure units (glass plates) and the reproduction rate of surviving mites was then evaluated from day 7 until day 14 after treatment by counting the total number of offspring (eggs and larvae) produced.

Findings

The mortality / escaping rate in the control group up to day 7 after treatment was 11.0%. The mean corrected mortality of the mites, and the mean reproduction rate of the surviving females exposed to the test item and the toxic reference is given below:

Mortality / Reproduction - 7 days after treatment

Test item		Deltamethrin EW 15 g/L					
Test organism		*Typhlodromus pyri*					
Exposure on		Detached apple leaves (day 0 to day 7 after application)					
		Mortality after 7 days [%]			Reproduction		
Treatment	mg a.s./ha	Uncorr.	Corr.	P-value (*)	Rate (eggs per female)	Red. rel. to Control [%]	P-value (#)
Control	0	11.0	-	-	8.2	-	-
Test item	2.5	22.0	12.4	0.028 sign.	7.9	3.7	0.410 n.sign.
Test item	5.5	30.0	21.3	<0.001 sign.	6.9	16.3	0.364 n.sign.
Test item	11.2	53.0	47.2	<0.001 sign.	5.9	27.4	0.004 sign.
Test item	23.6	55.0	49.4	<0.001 sign.	4.6	44.0	0.019 sign.
Test item	50.0	83.0	80.9	<0.001 sign.	n.a.	n.a.	
Reference item	15 g a.s./ha	72.2	68.8		n.a.	n.a.	

LR_{50}: 16.5 mg a.s./ha; 95 % Confidence Interval: 10.0 - 25.1; calculated with Probit analysis
* Fisher`s Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
# Wilcoxon test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed; n.sign. not significant; sign. significant

Conclusion

In this extended laboratory test the effects of Deltamethrin EW 15 g/L residues on the survival of the predatory mite *Typhlodromus pyri* were determined at the rates of 2.5, 5.5, 11.2, 23.6 and 50.0 mg as/ha applied to detached apple leaves.

At the test item rates of 2.5 and 5.5 mg as/ha a corrected mortality of 12.4% and 21.3% has been observed. 47.2% and 49.4% corrected mortality, respectively, occurred in the 11.2 and 23.6 mg as/ha rate. In the highest rate of 50.0 mg as/ha the corrected mortality was 80.9%.

The LR_{50} was calculated to be 16.5 mg as/ha.

At 2.5, 5.5, 11.2 and 23.6 mg as/ha the reproduction was reduced by 3.7%, 16.3%, 27.4% and 44.0%, respectively.

The figures obtained fulfill the validity criteria of the laboratory method for exposure on glass plates.

*****

Report:	**KCP 10.3.2.2/02, [redacted] (2011)**
Title:	Toxicity to the predatory mite Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test with aged residues on apple Deltamethrin EW 15 g/L
Document No.:	M-419712-01-1 (Rep. No.:CW11/042)
Guidelines:	Blümel et al. (2000) modified: Use of treated apple plants; mites exposed to freshly applied and under semi-field conditions aged residues on detached leaves enclosed in ventilated cells (Munger cages) for the first 7 days of each bioassay; Candolfi et al. (2001).
GLP	GLP study

Material and Methods

The oil in water emulsion Deltamethrin EW 15 g/L was tested, specified by sample description: TOX08992-00; specification no.: 102000013165 - 05; batch ID: 2010-002975 [analysed content of active ingredient: Deltamethrin 15.35 g/L]; density: 1.023 g/mL.

The test item was applied two times with 12.5 g as/ha diluted in 400 L deionised water/ha on potted apple plants (*Malus sylvestris*). The application interval in between was 7 days. The control was treated with deionised water in the same way as the test item.

The toxic reference Dimethoate was applied at 15 g as/ha diluted in 400 L deionised water/ha on the day of the second application on potted apple plants as well. For the further exposure dates it was applied directly on detached apple leaves (with 15 g as/ha diluted in 200 L deionised water/ha). It was included to indicate the relative susceptibility of the test organisms and the test system.

Aging of the spray deposits of the test item on the potted apple plants took place under semi-field conditions with UV permeable rain protection during the first four weeks of the study. Five bioassays were performed, the first started on the day of the second application (0DAT2 = 0 days after treatment 2) and the last one eight weeks later (56DAT2).

Predatory mites (*Typhlodromus pyri*) were exposed to these residues on the treated leaf surfaces. Mortality of 100 predatory mites, protonymphs at study start (10 replicates with 10 individuals per test group) was assessed 7 days after exposure in all bioassays and up to 14 days in the fourth and fifth bioassay by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

Due to the known repellent effects of the test item, the mortality part of each bioassay was performed in closed but actively ventilated cells (Munger cages). On day 7 after the start of the fourth and fifth bioassay, the surviving mites were transferred on untreated open exposure units (glass plates) and the reproduction rate of surviving mites was then evaluated from day 7 until day 14 after treatment by counting the total number of offspring (eggs and larvae) produced.

From these data the endpoints mortality (after 7 days) and effects on reproduction were calculated and summarized on next page.

Findings:

Test item:	**Deltamethrin EW 15 g/L**				
Application:	2 x 12.5 g as/ha (interval of 7 days)				
Test organism:	*Typhlodromus pyri*				
Exposure on:	Dried spray deposits on apple leaves from treated apple plants (day 0 to day 7 after start of the bioassay)				
Start bioassay:	**0DAT2**[a] (0 weeks)	**14DAT2**[a] (2 weeks)	**28DAT2**[a] (4 weeks)	**42DAT2**[a] (6 weeks)	**56DAT2**[a] (8 weeks)
	Mortality (%) after 7 days				
Control:	7.0	11.0	8.0	4.0	9.0
Test item:	100.0	100.0	93.0	44.0	10.0
Reference item:	75.0	89.0	87.0	70.0	65.0
	Corrected Mortality (%)				
Test item:	**100.0** (p-value < 0.001, sign.[b])	**100.0** (p-value < 0.001, sign.[b])	**92.4** (p-value < 0.001, sign.[b])	**41.7** (p-value < 0.001, sign.[b])	**1.1** (p-value 0.500, n.sign.[b])
Reference item:	73.1	87.6	85.9	68.8	61.5
	Reproduction				
	Number of eggs per female				
Control:	n.a.	n.a.	n.a.	**7.4**	**7.1**
Test item:	n.a.	n.a.	n.a.	**4.0**	**7.0**
	Reduction rel. to control (%)				
Test item:	n.a.	n.a.	n.a.	**46.6** (p-value 0.009, sign.[c])	**0.3** (p-value 0.470, n.sign.[d])

[a] Days after second treatment
[b] Fisher´s Exact test (one-sided); p-values adjusted according to Bonferroni-Holm
[c] one-way ANOVA, Williams test (one-sided)
[d] Wilcoxon test (one-sided), p-values adjusted according to Bonferroni-Holm
n.a. = not assessed; sign. = significant; n.sign. = not significant

Conclusions:

In this extended laboratory test the effects of Deltamethrin EW 15 g/L residues (aged under semi-field conditions, with rain protection during the first four weeks of the study) on the survival of the predatory mite *Typhlodromus pyri* were determined after two applications of 12.5 g as/ha with an application interval of 7 days onto apple plants (*Malus sylvestris*).
In the first 4 weeks (28 days) after the second application all bioassays resulted in a corrected mortality >92%.
After 6 weeks (42 days) of aging of the test item residues, the effects on mortality decreased to 41.7% corrected mortality and an assessment of the reproductive performance was performed which resulted in 46.6% reduction of reproduction compared to the control.
In the bioassay starting 8 weeks after the last application (56DAT2), a corrected mortality of 1.1% was found and the reduction in reproduction was 0.3%.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

*****

Report:	**KCP 10.3.2.2/03, [redacted] (2011)**
Title:	Toxicity to the parasitoid wasp *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae) using an extended laboratory test on barley Deltamethrin EW 15 g/L
Document No.:	M-400499-01-1 (Rep. No.:CW10/082)
Guidelines:	Mead-Briggs et al. (2000), Mead-Briggs et al. (2009), Candolfi et al. (2001)
GLP	GLP study

Material and methods:

An oil in water emulsion formulation of Deltamethrin EW 15 g/L was tested, specified by sample description: TOX 08992-00; specification no.: 102000013165-05; batch ID: 2010-002975 [analysed content of active ingredient: Deltamethrin 15.35 g/L]; density 1.023 g/mL.

The test item was applied on barley seedlings at rates of 0.25, 0.44, 0.79, 1.41 and 2.50 g as/ha and the effects on the parasitoid wasp *Aphidius rhopalosiphi* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 30 g as/ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 30 females (6 replicates with 5 wasps per test group) was assessed 2, 24 and 48 h after exposure.

Repellency of the test item was assessed during the initial 3 hours after the release of the females. Five separate observations were made at 30-minute intervals starting 15 minutes after the introduction of all wasps.

From the water control and the dose rates of 0.25, 0.44, 0.79 and 1.41 g as/ha, 15 impartially chosen females per treatment were each transferred to a cylinder containing untreated barley seedlings infested with *Rhopalosiphum padi* for a period of 24 hours. The number of mummies was assessed 11 days later.

Findings:

Test item		Deltamethrin EW 15 g/L						
Test organism		*Aphidius rhopalosiphi*						
Exposure on		Barley seedlings						
		Mortality after 7 days [%]			Reproduction		Repellency	
Treatment	g a.s./ha	Uncorr.	Corr.	P-value (*)	Rate (mummies per female)	Red. rel. to Control [%] P-value (#)	% Wasps on plant	Red. rel. to Control [%] P-value (##)
Control	0	0.0	-	-	44.1	-	48.7	
Test item	0.25	3.3	3.3	1.000 n.sign.	30.4	31.3 0.079 n.sign.	45.0	7.5 0.132 n.sign.
Test item	0.44	3.3	3.3	1.000 n.sign.	34.9	21.0 0.080 n.sign.	52.3	7.5 0.157 n.sign.
Test item	0.79	0.0	0.0	1.000 n.sign.	36.7	16.8 0.145 n.sign.	39.3	19.2 0.166 n.sign.
Test item	1.42	20.0	20.0	0.047 sign.	32.5	26.3 0.081 n.sign.	31.5	35.3 0.173 n.sign.
Test item	2.50	90.0	90.0	0.001 sign.	n.a.	n.a.	36.8	24.4 0.177 n.sign.
Reference item	3.0	90.0	90.0		n.a.	n.a.	49.7	-2.1

LR_{50}: 1.79 g as/ha; 95% Confidence Interval (1.59 - 2.00) calculated with Probit analysis
* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
# Wilcoxon test (one-sided), p-values are adjusted according to Bonferroni-Holm
one-way ANOVA, Williams test (one-sided)
n.a. not assessed, n.sign. not significant, sign. significant

Conclusion:

In this extended laboratory test the effects of residues of Deltamethrin EW 15 g/L on the survival of *Aphidius rhopalosiphi* were determined at 0.25, 0.44, 0.79, 1.41 and 2.50 g as/ha, applied to barley seedlings.

In the test item rates of 0.25 and 0.44 g as/ha 3.3% corrected mortality was observed. At the rate of 0.79 g as/ha no mortality was detected and 20.0% and 90.0% in the 1.41 and 2.50 g as/ha rate. The LR50 was calculated to be 1.79 g as/ha.

No dose related repellent effect of the test item was observed.

The reduction in reproductive success relative to the control at the 0.25, 0.44, 0.79 and 1.41 g as/ha rate was 31.1%, 21.0%, 16.8% and 26.3%, respectively.

The figures obtained fulfill the validity criteria of the extended laboratory method (Mead-Briggs et al., 2009).

*****

Report:	**KCP 10.3.2.2/04, [redacted] (2011)**
Title:	Toxicity to the ladybird beetle *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) using an extended laboratory test on *Malus sylvestris* Deltamethrin EW 15 g/L
Document No.:	M-401570-01-1 (Rep. No.: CW10/081)
Guidelines:	Schmuck et al. (2000) modified, Candolfi et al. (2001)
GLP	GLP study

Materials and methods

The oil in water emulsion formulation Deltamethrin EW 15 g/L was tested, specified by sample description: TOX 08992-00; specification no.: 102000013165-05; batch ID: 2010-002975 [analysed content of active ingredient: Deltamethrin 15.35 g/L], density: 1.023 g/mL.
The test item was applied to detached leaves of *Malus sylvestris* at rates of 8, 16, 32, 63 and 125 mg as/ha and the effects on the ladybird beetle *Coccinella septempunctata* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 12 g as/ha was included to indicate the relative susceptibility of the test organisms and the test system.
The preimaginal mortality of 40 larvae was assessed till the hatch of the imagines (up to 15 days). The fertility and fecundity of the surviving hatched adults were then evaluated over the period of 17 days.

Findings

Test item		Deltamethrin EW 15 g/L				
Test organism		*Coccinella septempunctata*				
Exposure on		Detached apple leaves				
		Mortality [%]			Reproduction	
Treatment	mg a.s./ha	Uncorr.	Corr.	P-value(**)	Fertile eggs per female and day	Fertility [hatching rate in %]
Control	0	20.0	-	-	11.1	88.6
Test item	8	20.0	0.0	0.610 n.sign.	12.6	78.2
Test item	16	50.0	37.5	0.009 sign.	23.8	93.6
Test item	32	67.5	59.4	<0.001 sign.	18.9	64.4
Test item	63	70.0	62.5	<0.001 sign.	n.a.	n.a.
Test item	125	92.5	90.6	<0.001 sign.	n.a.	n.a.
Reference item	12 g a.s./ha	100.0	100.0	-	n.a.	n.a.

LR_{50}: 29.8 mg as/ha; 95 % Confidence Interval: (18.9 - 42.1) (calculated with Probit analysis)
* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm n.a. not assessed n.sign. not significant sign. significant

Conclusion

In this extended laboratory study the effects of the test item residues of Deltamethrin EW 15 g/L to larvae of the ladybird beetle *Coccinella septempunctata* were determined. The application was done onto detached leaves of *Malus sylvestris*.

The test item rate of 8 mg as/ha had no influence on preimaginal mortality. At the rates of 16 and 32 mg as/ha, a corrected mortality of 37.5% and 59.4%, respectively, occurred. In the highest rates of 63 and 125 mg as/ha, corrected preimaginal mortalities of 62.5% and 90.6% were found.

The LR_{50} was calculated to be 29.8 mg as/ha.

Reproduction was assessed in the three lowest test rates of Deltamethrin EW 15 g/L, 8, 16 and 32 mg as/ha. The mean number of fertile eggs per female and day was 11.1 in the control and 12.6, 23.8 and 18.9, respectively, in the 8, 16 and 32 mg as/ha rate. Because the reproductive performance was within the historical data base for control beetles (≥ 2 fertile eggs per female and day, Schmuck et al. 2000) this parameter is considered as not affected at these test item rates.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates.

*****

Report:	**KCP 10.3.2.2/05, [redacted] (2011)**
Title:	Toxicity to the green lacewing *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) using an extended laboratory test on *Malus sylvestris* Deltamethrin EW 15 g/L
Document No.:	M-400889-01-1 (Rep. No.: CW10/085)
Guidelines:	Vogt et al. (2000) modified, Candolfi et al. (2001)
GLP	GLP study

Materials and methods

The oil in water emulsion formulation of Deltamethrin EW 15 g/L was tested, specified by sample description: TOX 08992-00; specification no.: 102000013165-05; batch ID: 2010-002975 [analysed content of active ingredient: Deltamethrin 15.35 g/L]; density: 1.023 g/mL.

The test item was applied to detached apple leaves at rates of 0.25, 0.59, 1.37, 3.20 and 7.50 g as/ha and the effects on the green lacewing *Chrysoperla carnea* were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 12.0 g as/ha was included to indicate the relative susceptibility of the test organisms and the test system.

The preimaginal mortality of 40 larvae was assessed till the hatch of the imagines (up to 20 days). The fertility and fecundity of the surviving hatched adults were then evaluated over the period of one week.

Findings

Test item		Deltamethrin EW 15 g/L				
Test organism		*Chrysoperla carnea*				
Exposure on		Detached apple leaves				
		Mortality [%]			Reproduction	
Treatment	g a.s./ha	Uncorr.	Corr.	P-value (*)	Eggs per female and day	Fertility [hatching rate in %]
Control	0	7.5	-	-	16.9	82.1
Test item	0.25	7.5	0.0	1.000 n.sign.	22.8	79.8
Test item	0.59	2.5	-5.4	1.000 n.sign.	31.0	85.5
Test item	1.37	10.0	2.7	1.000 n.sign.	23.8	81.4
Test item	3.20	7.5	0.0	1.000 n.sign.	27.8	90.2
Test item	7.50	37.5	32.4	0.006 sign.	17.4	85.2
Reference item	12.0	92.5	91.9	-	n.c.	n.a.

LR_{50} > 7.50 g as/ha
* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.a. not assessed, n.sign. not significant, sign. significant

Conclusion

In this extended laboratory study the effects of the test item residues to larvae of the green lacewing *Chrysoperla carnea* were determined; the application was made onto detached apple leaves.
The test item rates of 0.25, 0.59, 1.37 and 3.20 g as/ha had no or only minor influence on mortality. At the highest test item rate of 7.50 g as/ha a corrected mortality of 32.4% occurred which was statistically significant different compared to the control. The LR_{50} was estimated to be >7.50 g as/ha.
There were no adverse effects of the test item on the reproductive performance at all rates tested. The mean number of eggs/female/day was above the lower limit given as validity criterion for the glass plate method (mean number of eggs/female/day: ≥ 15, mean hatching rate: ≥ 70 %) according to the historical database of the ring testing group (Vogt, H. et al., 2000).
The figures obtained fulfil the validity criteria of the laboratory method for the exposure on glass plates.

*****

Supplemental information from the literature

Report:	**KCP 10.3.2.2/06; Broufas, G. D.; Pappas, M. L.; Vassiliou, G.; Koveos, D. S.; Editor(S): Vogt, H.; Jansen, J. P.; Vinuela, E.; Medina, P. (2008)**
Title:	Toxicity of certain pesticides to the predatory mite Euseius finlandicus (Acari: Phytoseiidae).
Source:	IOBC/WPRS Bulletin 35, p. 85-91
DOI No: Document No:	- M-461231-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The acute and residual toxicity of certain used pesticides in plum orchards in Greece to the predatory mite *Euseius finlandicus* were determined with laboratory and semi-field experiments. Material and methods as well as results are summarized here only for deltamethrin.
The test concentration in both experiments was 0.75 g a.i./hL Decis EC 2.5. The acute toxicity was evaluated under laboratory conditions (25°C and a photoperiod of 16:8 LD) using detached bean leaf disks (4 cm in diameter) which were sprayed with a Potter spraying tower calibrated to approximately 2 mg wet deposit per cm^2 [200 L/ha]. Plants sprayed with deionized water were used as the control group. 15 Protonymphs of *E. finlandicus* were transferred on the sprayed leaf disks and mortality was recorded every day. Cumulative mortality was assessed after 7 days to the spray residues. Mortality percentages were adjusted for the control mortality using Abbott's formula[29]. Fecundity of the surviving females was assessed from 7th to 14th day following the spray application and mean cumulative number of eggs per female was calculated as described by Blümel et al. (2002)[30]. Additionally, the total effect values (E) were calculated, according to Overmeer & Van Zon (1982)[31].
In a second group of experiments the persistence of the pesticides was assessed. Three years old plum potted trees (cv Vanilia), were sprayed till run off with a hand sprayer and subsequently maintained in the field. Control trees were sprayed with dionized water. At certain time intervals (i.e. 3, 7, 10, 15, 20 and 25 days) following spray application, leaves were cut from the trees and transferred to the laboratory. On each leaf were placed 15 protonymphs of the predatory mite with some *Typha* sp. pollen as food (25°C and a photoperiod of 16:8 LD). Mortality and egg production of the surviving mites were scored, as described above for the laboratory bioassays.
For the laboratory test, deltamethrin caused 100% mortality. Thus, no fecundity could be tested. The semi-field experiment indicated that the effects from deltamethrin residues aged for 20, 25, and 30 days decreased to 42.1%, 28.8%, and 10.0%, respectively.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Decis EC 2.5
Active substance(s): Deltamethrin

[29] Abbott. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
[30] Blümel et al. 2002. 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/WPRS
[31] Overmeer & Van Zon. 1982. A standardized method for testing the side effects of pesticides on the predacious mite Amblyseius potentillae (Acari: Phytoseiidae). Entomophaga 27: 357-364.

Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Euseius finlandicus*
Cultivar:	-
Source of test species:	Two months old stock colony from commercial plum orchard (Alexandria, Northern Greece)
Age of test organisms at study initiation	Protonymph
Crop growth stage at treatment:	
Holding conditions prior to test:	25°C and a photoperiod of 16:8 LD
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Extended laboratory and aged residue test (semi-field)
Duration of study:	14 days
Treatments:	*Laboratory:* Deltamethrin (Decis EC 2.5) and control (deionized water) *Semi-field bioassay:* 0, 3, 7, 10, 15, 20, 25 and 30 days aged deltamethrin (Decis EC 2.5) residues and control (deionized water)
Test concentrations	*Laboratory:* 0.75 g/hl; 2 mg/cm² deposit *Semi-field bioassay:* 0.75 g/hl; until run off
Number of replicates:	10 replicates
Individuals per replicate:	15 individuals
Test units (type and size):	*Laboratory:* 4 cm in diameter leaf disc placed upside down on wet cotton wool inside a plastic Petri dish (5 cm in diameter) *Semi-field bioassay:* leaf disc placed upside down on wet cotton wool inside a plastic Petri dish (5 cm in diameter)
Application / device / nozzles:	*Laboratory:* Potter Precision Tower (Burkard Manufacturer®, Rickmansworth, UK) (2 mg/cm² deposit) *Semi-field bioassay*: hand sprayer (until run off)
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	*Laboratory bioassay:* bean leaf disks *Semi-field bioassay:* leafs of three years old plum potted trees (cv Vanilla)
Temperature / relative humidity:	25°C
Photoperiod:	16:8 LD
Lighting	-

pH:	-
Organic matter (C_{org}):	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality and fecundity
Measurement frequency:	Mortality was assessed daily and fecundity was assessed at the end of the experiments
Statistical analyses:	

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

For the laboratory test, deltamethrin caused 100% mortality. Thus, no fecundity could be tested. The semi-field experiment indicated that aged deltamethrin residues reduced the toxicity.

Table 1: Toxicity of deltamethrin to the predatory mite E. finlandius (extended laboratory tests)

Pesticide	* E (%) after							
	0	3	7	10	15	20	25	30
	days following application							
Deltamethrin	M	M	M	83.1	81.5	42.1	28.8	10.0

M= 100% mortality * E= overall effect

In the laboratory test on leaves, deltamethrin [1.5 g a.i./ha] caused 100% mortality within 7 days. The semi-field experiment indicated that the effects from deltamethrin residues aged decreased down to 10% after 30 days.

Comment by the Notifier

The publication confirms the known toxicity of deltamethrin to predatory mites (100% mortality at 1.5 g a.s./ha) and supports the results of the regulatory aged residue studies that indicate the potential for recovery within a few weeks. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.2/07; BUNDSCHUH, R.; SCHMITZ, J.; BUNDSCHUH, M.; ALBRECHT [redacted] (2012)**
Title:	Does insecticide drift adversely affect grasshoppers (orthoptera: saltatoria) in field margins? A case study combining laboratory acute toxicity testing with field monitoring

	data
Source:	Environmental Toxicology and Chemistry, Vol. 31, No. 8, pp. 1874–1879
DOI No.: Document No.:	10.1002/etc.1895 M-462168-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The present study assessed effects of five of insecticides deltamethrin on the survival of *Chorthippus sp.* grasshopper nymphs by considering two routes of exposure (contact and oral). The laboratory toxicity tests revealed a sensitivity of grasshoppers with regard to the insecticides tested in the present study similar to that of the standard test species used in arthropod risk assessments.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis
Active substance(s):	Deltamethrin (25 g/L)
Adjuvant / Surfactant:	n/a
Source of test item:	[redacted]
Lot/Batch number:	n/a
Purity:	n/a
Storage conditions:	n/a

2. Test solutions

Vehicle/solvent:	n/a
Source of vehicle/solvent:	n/a
Concentration of vehicle/solvent:	n/a

3. Test organism(s)

Species:	*Chorthippus sp.* (*Chorthippus parallelus* and *Chorthippus dorsatus*)
Cultivar:	
Source of test species:	sampled from grassland [redacted] Germany [redacted]
Age of test organisms at study initiation /	first instar nymphs
Crop growth stage at treatment:	
Holding conditions prior to test:	n/a
Acclimatisation:	In the laboratory all individuals were kept together for at least 12 h under a natural light/dark rhythm in a terrarium covered with a mesh screen (white double yarn, 1-mm mesh size; Windhager) for continuous aeration.

B. Study design and methods

1. Test procedure	*Spraying procedure and toxicity test*
Test system (study type):	semi-field conditions
Duration of study:	48 h
Treatments:	Three treatments (contact exposure; oral exposure; contact and oral exposure)
Test concentrations	Five concentration and control
Number of replicates:	Six replicates; ten control replicates
Individuals per replicate:	five
Test units (type and size):	n/a
Application / device / nozzles:	Air assisted experimental sprayer (Schachtner Gerätetechnik) equipped with four 110° flat-fan ‘‘TeeJet’’ nozzles (XR 11002-VS; Schachtner Gerätetechnik) was used to spray the respective insecticide concentration onto the surface of the test vessels or the pots with the grass mixtures.
Water volume:	
Calibration of sprayer:	The nozzles were integrated in a fume cupboard with a distance of 0.25 m between the nozzles and at a height of 0.7 m above the application area), calibrated to meet the recommended application rate of arable crops of 400 l/ha and cover the surface of the vessels and grass mixtures with a homogeneous spray deposit. For calibration, test vessels were sprayed with distilled water and then weighed to ensure the desired application rate.
2. Environmental conditions	
Test medium:	n/a
Temperature / relative humidity:	21.0°C (+/- 5.2 SD)/ 62.6% (+/-24.0 SD)
Photoperiod:	n/a
Lighting	n/a
pH:	n/a
Organic matter (C_{org}):	n/a
$CaCO_3$	n/a
Cation exchange capacity:	n/a
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	n/a
Fertilization:	n/a
3. Observations and measurements:	
Analytical parameters measured:	n/a
Biological parameters measured:	Effects on the test organisms were recorded after 48 h and categorized as ‘‘alive’’ without any observable effects or with constrained movements and ‘‘lethal.’’
Measurement frequency:	
Statistical analyses:	The statistical analyses were performed using the software R. The 48-h LR50 (the application rate of an insecticide causing 50% mortality of the test organisms) values determined during the toxicity studies were calculated with dose–response models using the ‘‘drc’’ package. For each toxicity test, the model fitting the data best, based on Akaike’s information criterion, was chosen, that is, Log-logistic or Weibull models. The LR50 values were based on the insecticide field

application rate (g a.i./ha); the dose received by the individual grasshopper was not measured.
Two- and three-factorial analyses

RESULTS AND DISCUSSION

Validity criteria:

The control mortality did not exceed 10%.

Biological findings:

Toxicity was highest for the contact exposure scenario, the combination of contact and oral exposure mostly displayed lower toxicities.

Table 3. LR_{50} values of *Chorthippus sp.* after 48 h of exposure to the insecticides investigated, the model used for calculation, and the LR_{50} values of *A. rhopalosiphi* and *T. pyri* from the literature

a.i.	Exposure scenario	Model	*Chorthippus sp.*		
			LR_{50} 48 h (g a.i./ha)	Lower 95% CI	Upper 95% CI
Deltamethrin	Contact	Log-logit	0.10	0.07	0.13
	Contact and oral	Weibull	0.21	0.13	0.29
	oral	Log-logit	0.82	0.53	1.12

LR_{50} = application rate of an insecticide causing 50% mortality of the test organisms; CI = confidence interval; NC = no calculation possible; a.i. = active ingredient.

Chorthippus sp.
LR50 (48 h) = 0.10 g a.i./ha (contact exposure, on plastic substrate)
LR50 (48 h) = 0.21 g a.i./ha (contact and oral exposure)
LR50 (48 h) = 0.82 g a.i./ha (oral exposure)

Comment by the Notifier

The publication confirms the known toxicity of deltamethrin to herbivorous insects. Since the study results indicate a lower sensitivity of the tested taxon compared to the non-target arthropods tested for the regulatory data package the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.2/08; M.Z. da Silva, M.E. Sato, C.A.L. de Oliveira, B. Veronez (2012)**
Title:	Toxicity of agrochemicals to the citrus leprosis mite *Brevipalpus phoenicis* (Geijskes) and to the predatory mite *Neoseiulus californicus* (McGregor) (Acari: Tenuipalpidae, Phytoseiidae)
Source:	Arq. Inst. Biol., São Paulo, v.79, n.3, p.363-370, Jul.
DOI No.:	none
Document No.:	M-462298-01-2
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The objective of this study was to evaluate various pesticides used in citrus crops in regard to their toxicity to *Brevipalpus phoenicis* and *Neoseiulus californicus*, a potential predator of phytophagous

mites in the crop. Bioassays were conducted using orange-tree leaf discs for *N. californicus* and fruits for *B. phoenicis*. *N. californicus* was tested with 35 adult females per leaf disc. *B. phoenicis* was tested 50 adult females per test unit. The applications were made using a Potter tower, with 4 replications for each treatment. Assessments of the number of live and dead mites were observed 72 hours after application. Deltamethrin was observed as harmless to *N. californicus*. And in addition, Deltamethrin was not effective in controlling *B. phoenicis*, too.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 2,5 EC
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	None
Source of test item:	Not reported
Lot/Batch number:	Not reported
Purity:	Not reported

2. Test organism(s)

Species:	*Brevipalpus phoenicis* (Geijskes) and *Neoseiulus californicus* (McGregor)
Source of test species:	*B. phoenicis:* citrus orchard in the municipal district of Piracicaba, SP *N. californicus:* collected from a commercial strawberry plantation in the municipal district of Atibaia, SP.
Age of test organisms at study initiation:	adult mites - 5 days old
Holding conditions prior to test:	The predatory mites were bred on jackbean plants, *Canavalia ensiformis* (L.), grown in 500 ml plastic containers, fed on *Tetranychus urticae* Koch (Tetranychidae). The population was then transferred to discs of the Pera variety orange-tree leaves, 8 cm in diameter, placed on a layer of hydrophilic cotton wool, kept saturated with distilled water, in a Petri dish (15 cm in diameter). To prevent the mites escaping, the edge of the leaf was kept covered with moist cotton wool. *T. urticaei* eggs, nymphs and adults and pollen of the castor oil plant, *Ricinus communis* L., were placed in abundance on each disc as food for the predatory mites.
Acclimatisation:	

B. Study design and methods

1. Test procedure

Test system (study type):	according to the method described by KNIGHT et al. (1990)
Duration of study:	72 h
Treatments:	One treatment
Test concentrations	50 mL/100 L equivalent to 1.25 g a.s./100 L [1.6 mg/cm2 equivalent to 2 g a.s./ha]
Number of replicates:	four replications
Individuals in total:	Thirty-five adult of *N. californicus* and 50 adult females of *B. phoenicis*
Test units (type and size):	Petri dish (9 cm in diameter) for *N. californicus* and an area of

	approximately 3 cm2 encircled by adhesive tape (Tanglefoot®) on a fruit for *B. phoenicis*
Application / device / nozzles:	sprayed using a Potter spray tower (Burkard Scientific, Uxbridge, UK),
Water volume:	spray volume of 2 mL
Calibration of sprayer:	calibrated at 68.9 kPa to 1.6 mg/cm2

2. Environmental conditions

Test medium:	Hydrophilic cotton wool for *N. californicus* and fruit for *B. phoenicis*
Temperature / relative humidity:	25 ± 2ºC, 70 ± 10%
Photoperiod:	12 hours

3. Observations and measurements:

Analytical parameters measured:	None
Biological parameters measured:	Mortality
Measurement frequency:	Once 72 h after application
Statistical analyses:	analysis of variance (ANOVA)

RESULTS AND DISCUSSION

Validity criteria:
Not stated

Biological findings:
No mortality (%) occurred in the treatment with Deltamethrin for *N. californicus*. In addition, *B. phoenicis* mortality rate (%) was not statistically different to the control treatment (2%) (table 1). Therefore Deltamethrin was observed as harmless for both species.

Table 1: Percentage mortality of *Brevipalpus phoenicis* adult females and *Neoseiulus californicus* adult females observed 72 hours after product application

Technical name	Dosage		Mortality (%)[1]	
	per 100 L	At m 100 L	*B. phoenicis*	*N. californicus*
Deltamethrin	50.0 mL	1.25 g	2.00 A	0.00 A
Control	distilled water		2.00 A	0.00 A

[1]Means followed by the same letter in the column do not differ from one another using Tukey's test (p < 0.05)

Assessments of the number of live and dead mites were observed 72 hours after application. Deltamethrin was observed as harmless to *N. californicus* (0% Mortality). The mortality rate for *B. phoenicis* was 2 % like in the control treatment.

Comment by the Notifier
B. phoenicis is a pest species and therefore not considered relevant for the risk assessment. The data on the predatory mite *N. californicus* indicate a low susceptibility of this species compared to T. pyri that has been tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	KCP 10.3.2.2/09; Silva, M. Z. Da; Oliveira, C. A. L. De; Sato (2009)
Title:	Selectivity of the pesticides to the predaceous mite Agistemus brasiliensis Matioli, Ueckermann and Oliveira (Acari: Stigmaeidae). Seletividade de produtos fitossanitarios sobre o acaro predador Agistemus brasiliensis Matioli, Ueckermann and Oliveira (Acari: Stigmaeidae).
Source:	Revista Brasileira de Fruticultura, 31, 2, pp. 388-396
DOI No:	-
Document No:	M-462141-01-2
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The aim of this study was to evaluate the selectivity of the main pesticides with acaricide and/or insecticide activity used in citrus to *A. brasiliensis*. Material and methods as well as results are summarized for deltamethrin only.

A. brasiliensis (stigmaeid mites) used in this study were collected in August of 2005, from orange plants (Citrus sinensis) of the Pera and Valência varieties, from orchards located at the FCAV/UNESP campus in Jaboticabal, SP. They were bred on orange tree leaves of the Pera variety, placed on a layer of cotton wool in a Petri dish (15 cm diameter). Nymphs and adult of *B. phoenicis* and castor bean, *Ricinus communis* L., or bulrush, *Typha* sp., pollen were placed abundantly in each arena as food source. Holding conditions were 25 ± 2 °C, 70 ± 10% relative humidity and 12 h photophase.

Orange tree leaf disks (4 cm diameter) placed on a layer of cotton wool in 9 cm diameter Petri dishes containing 25 adult females of *A. brasiliensis* were sprayed with Deltamethrin (Decis 25 CE, 50 ml product/100 ml) using a Potter tower (2 ml solution, 1.6 mg/cm² deposit). The test was repeated 4 times. Test conditions were 25 ± 2 °C, 70 ± 10% RH and 12 h photophase. Mortality was assessed 72 hours after application. The evaluations were carried out using a stereomicroscope; predaceous mites unable to move a minimum distance equivalent to their body length when gently touched with a smooth hair paintbrush were considered dead.

Mortality of *A. brasiliensis* was subjected to analysis of variance, by the F-test, and the averages compared by Tukey's test. Furthermore, the effect of reproduction and the total or adverse effect were calculated.

The evaluation of the viability of the eggs laid on the arenas, between the application and 72 hours after the treatment, was carried out during a 7-day period.

Deltamethrin caused corrected mortalities in *A. brasiliensis* of 2.0% and did not affect oviposition and viability of the predator`s eggs.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 25 CE
Active substance(s):	Deltamethrin
Adjuvant, Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	-

Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Agistemus brasiliensis*
Cultivar:	-
Source of test species:	collected in August of 2005, from orange plants (Citrus sinensis) of the Pera and Valência varieties, from orchards located at the [redacted], SP
Age of test organisms at study initiation /	adults
Crop growth stage at treatment:	
Holding conditions prior to test:	25 ± 2 °C, 70 ± 10% relative humidity and 12 h photophase
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Extended bioassay on natural surface
Duration of study:	72 hours
Treatments:	Deltamethrin and Control
Test concentrations:	50 ml product/ 100 L
Number of replicates:	4 replicate
Individuals per replicate:	25 adults
Test conditions:	25 ± 2 °C, 70 ± 10% RH and 12 h photophase
Test units (type and size):	leaves from the citrus of the Pera variety (4 cm diameter) placed on a layer of cotton wool in 9 cm diameter Petri dishes.
Application / device / nozzles:	Potter tower
Water volume:	-
Calibration of sprayer:	68.9 kPa (1.6 mg/cm² deposit)

2. Environmental conditions

Test medium:	leaves from the citrus of the Pera variety (4 cm diameter)
Temperature / relative humidity:	25 ± 2°C, 70 ± 10% RH
Photoperiod:	12 h photophase
Lighting:	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality and egg viability
Measurement frequency:	72 h after application
Statistical analyses:	F-test and Tukey's test (5% probability)

RESULTS

Validity criteria:

No validity criteria were stated.

Biological findings:
Deltamethrin caused corrected mortalities in *A. brasiliensis* of 2.0% (±1.07%) and did not affect oviposition and viability of the predator`s eggs.

Comment by the Notifier
The data on the predatory mite *A. brasiliensis* indicate a low susceptibility of this species compared to *T. pyri* that has been tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.2/10; Silva, M. Z. Da; Oliveira, C. A. (2007)**
Title:	Residual toxicity of some pesticides recommended for citrus orchards on the predaceous mite Neoseiulus californicus (McGregor) (Acari: Phytoseiidae). Toxicidade residual de alguns agrotóxicos recomendados na citricultura sobre Neoseiulus californicus (McGregor) (Acari: Phytoseiidae).
Source:	Revista Brasileira de Fruticultura, 29, 1, pp. 85-90
DOI No: Document No:	- M-460948-01-2
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY
The aim of this study was to evaluate the residual toxicity of some of the main pesticides used in citrus orchards in Brazil on a population of *N. californicus* to obtain information about products which will not cause significant mortality of that predator. Material and methods as well as results are summarized only for deltamethrin.
The N. californicus population provided by [redacted] Laboratório de Entomolgia Econômica of the Centro Experimental Central, of the Instituto Biológico, Campinas – SP, was collected in a commercial culture of strawberries in the municipality of Atibaia – SP, on the 30th of September 1999.
N. californicus was kept on jack beans plants [Canavalia ensiformis (L.)] grown in 500 ml plastic pots in the laboratory and fed with *Tetranychus urticae* Koch, being transferred later to disks of "Pêra" orange tree leaves approximately 8 cm in diameter, and placed on a layer of cotton wool kept saturated with distilled water, in a Petri dish (15 cm diameter). . *T. surticae* nymphs and adults and castor beans pollen, *Ricinus communis* L., were placed on each arena in abundant quantity as the food source for the predaceous mites.
The culture was kept at 25 ± 2 °C, 70 ± 10% relative humidity and 12-hour photophase
For the residual toxicity test, 4 cm diameter disks of orange tree leaves of the "Pêra" variety were placed on top of a layer of cotton wool in a Petri dish (9 cm diameter). The cotton wool was kept permanently saturated with distilled water.
Deltamethrin (Decis 25 CE) spraying was carried out in a Potter tower applying 2 ml of solution at a pressure of 0.703 kg/cm². After two hours and 1, 3, 5, 7, 10, 14 and 21 days following the application, 10 *N. californicus* adult females were transferred to each arena. *T. urticae* nymphs and adults were supplied as food. Test conditions were 25 ± 2 °C, 70 ± 10% relative humidity and 12-hour photophase. Evaluation of the number of dead and living mites was carried out 72 hours after transfer to treated

arenas, using a stereomicroscope. Predaceous mites unable to move a minimum distance equivalent to their body length when touched gently with a smooth hair paintbrush were considered as dead. Analysis of variance of the number of living mites was assessed using the F-test and the means were compared by Tukey's test.
In the residual toxicity bioassay, deltamethrin did not cause significant mortalities to *N. californicus*, not being statistically different ($p>0.05$) from the control. Corrected mortalities were not higher than 4.2%.

A. Material

1. Test material

Test item:	Decis 25 CE
Active substance(s):	Deltamethrin
Chemical state and description:	-
Source of test item:	-
Batch number:	-
Purity:	-
Storage conditions:	-
Water solubility:	-

2. Test solutions

Vehicle/solvent:	-
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Neoseiulus californicus*
Cultivar:	-
Source of test species:	provided by [redacted] Laboratório de Entomolgia Econômica of the Centro Experimental Central, of the Instituto Biológico, Campinas – SP, was collected in a commercial culture of strawberries in the municipality of Atibaia – SP, on the 30th of September 1999.
Age of test organisms at study initiation:	Adult
Crop growth stage at treatment:	-
Holding conditions prior to test:	25 ± 2 °C, 70 ± 10% relative humidity and 12 hour photophase; *T. urticae* nymphs and adults and castor beans pollen, *Ricinus communis* L., in abundant quantity
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Residual toxicity study
Duration of study:	Periods form application to transfer of the test organisms: up to 21 days; Exposure time of the test organisms: 72 h
Treatments:	Exposure to two hours and 1, 3, 5, 7, 10, 14 and 21 days old deltamethrin residues on orange tree leave disks and control
Test concentrations	1.25 g a.i./ 100 L [1.5 mg/cm2 equivalent to 1.875 g a.s./ha]
Number of replicates:	5 replicates
Individuals per replicate:	10 adult females

Test conditions:	25 ± 2 °C, 70 ± 10% relative humidity and 12 hour photophase
Test units (type and size):	4 cm diameter disks of orange tree leaves of the "Pêra" variety were placed on top of a layer of cotton wool in a Petri dish (9 cm diameter). The cotton wool was kept permanently saturated with distilled water.
Application / device / nozzles:	Potter tower, 2 ml solution, pressure 0.703 kg/cm², 1.5 mg of solution per cm^2
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	4 cm diameter disks of orange tree leaves
Temperature / relative humidity:	25 ± 2 °C, 70 ± 10% relative humidity
Photoperiod:	12 hour photophase
Lighting	-
pH:	-
Organic matter (C_{org})	-
$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality
Measurement frequency:	72 h after transfer to treated arenas
Statistical analyses:	T-test and Tukey's test

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

In the residual toxicity bioassay, deltamethrin did not cause significant mortalities to *N. californicus*, not being statistically different ($p>0.05$) from the control. Corrected mortalities were not higher than 4.2%.

Table 1: Residual toxicity of pesticides to *N. californicus*, 72 hours after transfer of the mites to citrus leaves treated, after different periods from application to transfer: Percentages of population reduction.

Technical name	Period after application								
	Dosage g.a.i./100 L	2 hours	1 day	3 days	5 days	7 days	10 days	14 days	21 days
deltamethrin	1.25	4.2	2.2	0.0	0.0	0.0	0.0	0.0	0.0

In the residual toxicity bioassay, deltamethrin did not cause significant mortalities to *N. californicus*, not being statistically different (p>0.05) from the control. Mortalities were not higher than 4.2%.

Comment by the Notifier

The data on the predatory mite *N. californicus* indicate a low susceptibility of this species compared to *T. pyri* that has been tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.2/11; Desneux, N.; Fauvergue, X.; Dechaume-Moncharmont, F.X.; Kerhoas, L.; Ballanger, Y.; Kaiser, L. (2005)**
Title:	Diaeretiella rapae limits Myzus persicae populations after applications of deltamethrin in oilseed rape.
Source:	Journal of economic entomology, 98, 1 p. 9-17
DOI No: Document No:	10.1603/0022-0493-98.1.9 M-460865-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

This study investiagted the impact of *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) on populations of *M. persicae* when parasitoids were introduced on deltamethrin treated plants at increasing intervals after treatment.

Oilseed rape plants (two leaf stage;infested by M. persicae) were treated with 5 g ai/ha using a power-pack aerosol hand sprayer (T=0) (semifield). Then, the plants were divided into experimental groups of 30 plants each. The first two-level factor was the presence or absence of deltamethrin. The second two-level factor was the presence or absence of parasitoids (five males and five females released per plant). The four-level factor was the lag time between deltamethrin treatment and parasitoid release: parasitoids were released on the caged plants either 1 (T + 1), 2 (T + 2), 7 (T + 7), or 14 (T + 14) days after deltamethrin treatment.

To quantify subsequent aphid population dynamics, the number of aphids per plant was counted 7, 14, and 21 d after the introduction of parasitoids into the cage. Because all plants had been treated the same day, aphids were consequently counted on different calendar dates for each group, depending on the lag between deltamethrin treatment and parasitoid introduction. To evaluate *D. rapae* population dynamics, we collected mummies on the plants and counted the number of emerging females. This number provided an index, R0, of the net reproductive rate in the parasitoid population (i.e., the number of females produced per female and per generation).

Deltamethrin residues on the plant were analayzed 1, 2, 7 and 14 days after treatment. Between 27 and 34 leaves were sampled on several plants from each group. Hexane and dichloromethane were used to extract the residues. Resulting chemical samples were analysed by gas chromatography-mass spectrometry. The limit of detection was approx. 10 pg and the limit of quantification was approx. 20 pg.

Furthermore, the mortality rate of parasitoids when exposed to the four ages of deltamethrin residues on oilseed rape leaves (1, 2, 7 and 14 d after treatement; the same plants being used as for the analysis of residues) was evaluated under laboratory conditions. Untreated leaves were used as controls. 10 parasitoids were introduced and five repliactes were performed for each age of residues and the

controls. 24 h later, the dead parasitoids were counted. Insecticide exposure was performed at 20 ± 1°C, 65 ± 5% RH, and a photoperiod of 12:12 (L:D) h. This device contained untreated areas (i.e. plastic cage wall) similar to the semifield cages.
Second, we determined the toxicity of fresh deltamethrin residues without refuge areas. We used the formulated insecticide Decis micro. It was applied to cut leaves (7 cm in diameter) by using a Burgerjontype Potter-tower (Burgerjon 1956). Two different doses were tested: 0.5 g (AI)/ha and 5 g AI/ha; water-sprayed leaves were used as controls. Slightly modified exposure units developed by Jansen (1996)[32] were used. Ten parasitoids were introduced per unit. There were five replicates per dose and a control. After 24 h, the dead parasitoids were counted. Pesticide exposure was performed at 20 ± 1°C, 65 ± 5% RH, and a photoperiod of 12:12 (L:D) h.
First, both the pesticide and the parasitoid reduced aphid population growth, their effects were additive. Deltamethrin residues had no effect on the reproduction of *D. rapae* females. The total amount of extracted residues after 1 and 2 days was approx. 5 ng [AI]/cm² (corresponding to 0.5 g [AI]/ha). Seven and 14 d after insecticide treatment, little deltamethrin was found on the leaves (<0.5 ng (AI)/cm².
Furthermore, there was no mortality of parasitoids exposed to treated leaves in a device with a refuge area (over 14 days). In contrast, the 24 h mortality test without a refuge area showed 20% (dose of 0.5 g [AI]/ha) and 44.3% (dose of 5.0 g [AI]/ha) mortality. Both application doses induced significant parasitoid mortality compared with untreated leaves.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis micro
Active substance(s):	Deltamethrin
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test solutions

Vehicle/solvent:	Diluted water
Source of vehicle/solvent:	-
Concentration of vehicle/solvent:	-

3. Test organism(s)

Species:	*Diaeretiella rapae*, *Myzus persicae* and Oilseed rape plants
Cultivar:	-
Source of test species:	-
Age of test organisms at study initiation / Crop growth stage at treatment:	*Diaeretiella rapae*: 24-48 h and Oilseed rape plants: two-leaf stage
Holding conditions prior to test:	Mummified aphids containing *D. rapae* were removed from *Vicia fabae* L. leaves and stored in plastic petri dishes until parasitoid emergence. Adult females were mated at emergence and then held for 24 h in glass tubes (5 cm in length, 1 cm in diameter, five

[32] Jansen, J. P. 1996. Side effects of insecticides on Aphidius rhopalosiphi (Hym. Aphiddidae) in laboratory. Entomophaga 1: 37-43.

	individuals per tube), where they were supplied with a dilute honey solution (80%) but no aphids or plants.
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Semifield chronic effects and mortality on laboratory semifield treated leaves as well as mortality on laboratory treated leaves
Duration of study:	*Semifield:* 3 weeks; *Laboratory semifield treated leaves:* 24 days; *Laboratory treated leaves*: 24 h
Treatments:	Present and absence of *D. rapae*, Decis micro and control
Test concentrations	*Semifield* 5 g [AI]/ha; *Laboratory semifield treated leaves*: 5 g [AI]/ha; *Laboratory treated leaves*: 0.5 g [AI]/ha and 5 g [AI]/ha
Number of replicates:	*Semifield*: 2 by 2 by 4 factorial designs with repeated measures. The first two-level factor was the presence or absence of deltamethrin. The second two-level factor was the presence or absence of parasitoids (5 females and 5 males released per plant). The four-level factor was the lag time between deltamethrin treatment and parasitoid release. *Laboratory semifield treated leaves*: 5 replicates; *Laboratory treated leaves*: 5 replicates per dose
Individuals per replicate:	*Semifield:* 30 plants per group; *Laboratory semifield treated leaves*: 10 females; *Laboratory treated leaves*: 10 females
Test units (type and size):	*Semifield:* plastic cages (40 cm in height, 17 cm in diameter); *Laboratory semifield treated leaves*: a small aerated plastic cage (11.8 by 8.8 by 4.7 cm); *Laboratory treated leaves*: Slightly, modified exposure unit developed by Jansen (1996)[32]
Application / device / nozzles:	*Semifield:* power-pack aerosol hand sprayer (Nozzle: 30 psi, XR8001VS Teejet Spray, Teejet South Europe, Orléans, France) *Laboratory semifield treated leaves*: power-pack aerosol hand sprayer (Nozzle: 30 psi, XR8001VS Teejet Spray, Teejet South Europe, Orléans, France) *Laboratory treated leaves*: Burgerjontype Potter-tower
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Oilseed rape plants and leaves
Temperature / relative humidity:	*Semifield:* Outside conditions *Laboratory semifield treated leaves*: 20 ± 1°C, 65 ± 5% RH *Laboratory treated leaves*: 20 ± 1°C, 65 ± 5% RH
Photoperiod:	*Semifield:* Outside conditions *Laboratory semifield treated leaves*: 12:12 (L:D) h *Laboratory treated leaves*: 12:12 (L:D) h
Lighting	-
pH:	-
Organic matter (C_{org}):	-

$CaCO_3$	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	Deltamethrin concentration on leaves 1, 2, 7 and 14 days after application
Biological parameters measured:	Impact of Deltamethrin on Aphid Populations and Parasitoid Efficiency in Semifield Conditions; Impact of Deltamethrin on Net Reproductive Rate of Parasitoids; Toxicity of Deltamethrin-Sprayed Leaves (Device with Refuge Areas) and Toxicity of Deltamethrin-Sprayed Leaves (Device without Refuge Areas)
Measurement frequency:	Semifield: after 7, 14 and 21 d *Laboratory semifield treated leaves*: at test end (24 h) *Laboratory treated leaves*: at test end (24 h)
Statistical analyses:	linear model for repeated measure designs based on a Poisson distribution, a log-link function, and an exchangeable correlation matrix; type 2 functional response (Holling 1959)[33]; nonlinear regression based on the least squares method and an iterative procedure; Mann-Whitney test

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

First, both the pesticide and the parasitoid reduced aphid population growth, their effects were additive. Deltamethrin residues had no effect on the reproduction of *D. rapae* females. The total amount of extracted residues after 1 and 2 days was approx. 5 ng [AI]/cm² (corresponding to 0.5 g [AI]/ha). Seven and 14 d after insecticide treatment, little deltamethrin was found on the leaves (<0.5 ng (AI)/cm².

Furthermore, there was no mortality of parasitoids exposed to treated leaves in a device with a refuge area (over 14 days). In contrast, the 24 h mortality test without a refuge area showed 20% (dose of 0.5 g [AI]/ha) and 44.3% (dose of 5.0 g [AI]/ha) mortality. Both application doses induced significant parasitoid mortality compared with untreated leaves.

Comment by the Notifier

The data on the parasitoid *Diaeretiella rapae* indicate a slightly lower sensitivity compared to *A. rhopalosiphi* that has been tested for the regulatory data package. The fast dissipation of the residues is in line with the available DT50 data for deltamethrin. The aged residue part of the study confirms the known potential for recovery after initial effects. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

[33] Holling, C. S. 1959. Some characteristics of simple types of predation and parasitization. Can. Entomol. 91: 386Ð398.

Report:	KCP 10.3.2.2/12; Poletti, M.; Omoto, C. (2012)
Title:	Susceptibility to deltamethrin in the predatory mites *Neoseiulus californicus* and *Phytoseiulus macropilis* (Acari: Phytoseiidae) populations in protected ornamental crops in Brazil
Source:	Exp Appl Acarol (2012) 58:385–393
DOI No.:	10.1007/s10493-012-9588-z
Document No.	M-462290-01-1
Guidelines:	No
GLP:	No

EXECUTIVE SUMMARY

The objective of this research was to evaluate the susceptibility to deltamethrin in populations of the predatory mites *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) populations collected from protected ornamental crops in Brazil. The susceptibility to deltamethrin was characterized against immature and adult stages of both species. The immature and adult stages of *N.californicus* were approximately 3,600 and 3,000-fold more tolerant to deltamethrin than those of *P.macropilis*. The LC_{50} values for *N.californicus* were 866.3 mg a.i./L for immature stage and 970.1 mg a.i./L for adults. The LC_{50} values for *P.macropilis* were 0.2 mg a.i./L for immature stage and 0.3 mg a.i./L for adults. However, high variability in the susceptibility to this insecticide was detected among *P.macropilis* populations, with resistance ratios of up to 3,500-fold. LC_{50} values varied between 0.3 mg a.i./L and 1159.1 mg a.i./L.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis 25CE
Active substance(s):	25 g of deltamethrin l^{-1}
Adjuvant / Surfactant:	None
Source of test item:	[redacted]
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test organism(s)

Species:	*Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks)
Source of test species:	The population of *P. macropilis* used as the susceptible reference population (Pm) was collected in 2003, in a non-commercial bean crop located in Piracicaba, with no pesticide use. For studying intra-species variability to deltamethrin, we also collected populations of *P. macropilis* in commercial crops of roses (Pm2), gerbera (Pm3) and chrysanthemum (Pm4), in Holambra (Sa˜o Paulo State). The population of *N. californicus* (Nc) was obtained from the laboratory of Economic Entomology, Instituto Biolo´gico, Campinas, State of Sao Paulo. This population was collected in a commercial strawberry crop in Atibaia,

	Sa˜o Paulo State, in October 1999
Age of test organisms at study initiation	Immatures and adults
Holding conditions prior to test:	Predatory mites were kept on plants of *C. ensiformis* infested with *T. urticae*, used as a food source. Each species and the populations of *P. macropilis* were isolated in acclimatized rooms at 25 ± 2 °C, 70 ± 10 % relative humidity and 14 h photoperiod. Plants were replaced every 5 days to maintain predator populations, as described for the prey mites.

B. Study design and methods

1. Test procedure

Test system (study type):	Immatures
Duration of study:	48 h, 120 h and 7 d
Test concentrations:	For the effects on growth and susceptibility: Eggs laid within 24 h were sprayed with five logarithmically-distributed concentrations, ranging from 320 to 4,250 mg deltamethrin/l for *N. californicus* and from 0,056 to 1.8 mg deltamethrin/l for *P. macropilis*. For the variability in the susceptibility of adult females of *P.macropilis* females were sprayed with five concentrations of deltamethrin spaced in a log scale and ranging from 0.056 to 1.8 mg deltamethrin l-1 for the susceptible population (Pm) and from 56 to 3,200 mg deltamethrin l-1 for the other populations tested
Number of replicates:	Four to six repetitions were carried out for each concentration tested. Each replication consisted of four arenas containing five predatory mites.
Test units (type and size):	Petri dishes 3.5 cm in diameter
Application / device / nozzles:	Sprayed using a Potter spray tower (Burkard Manufacturing, Rickmansworth, Herts, England), set to a pressure of 10 psi. Each spray treatment consisted of 2 ml of suspension, depositing an average of approximately 1.50 ± 0.02 mg cm^{-2} [150 l/ha]

2. Environmental conditions

Test medium:	First disks of *C. ensiformis* leaf of 3 cm in diameter, infested with 20 adult females and 100 eggs of *T. urticae* were used and Half an hour after spraying, the cotton around the leaf disks was removed and each disk transferred onto an an agar–water mixture (3 %) in Petri dishes 3.5 cm in diameter.
Temperature / relative humidity:	25 ± 2 °C/ 70 ± 10 %
Photoperiod:	14 h

3. Observations and measurements:

Biological parameters measured:	Mortality was evaluated 120 h after direct spraying of the eggs and 48 h after spraying the adult females (→ LC_{50}-values)

Seven days after transferring the contaminated adult females onto the residue, the total number of predators (eggs, larvae, nymphs and adults) per arena was counted for each concentration. This data was used to estimate the instantaneous rate of increase (ri) (→NOEC and LOEC).

Measurement frequency: After 48 h, 120 h and after 7 days

Statistical analyses: Probit (LC_{50}) and ANOVA (NOEC/LOEC)

RESULTS AND DISCUSSION

Validity criteria:

No validity criteria were defined.

Findings:

Both immatures and adults of *N. californicus* were more tolerant to deltamethrin than *P. macropilis*. The tolerance ratios (TR) estimated for immatures and adults were approximately 3,600 times and 3,000 times respectively.

The highest concentration that did not affect the ri of *N. californicus* (NOEC) was 320 mg deltamethrin l-1 and the first concentration to cause a significant impact (LOEC) was 560 mg deltamethrin l-1.

LC_{50}-values are given in the tables below:

Table 1: Deltamethrin concentration–response of immature and adult stages of *Neoseiulus californicus* (Nc) and *Phytoseiulus macropilis* (Pm)

	Species	N (total no of mites used)	LC_{50} (mg a.i./L)	Slope +/- SE	X^2	df	Tolerance Ratio (=LC_{50} NC/LC_{50} Pm)
Immature	Nc	635	866.3	2.6 +/-0.2	16.6	3	3609.4
	Pm	428	0.2	1.8 +/-0.2	2.9	3	
Adult	Nc	1044	970.1	2.2 +/-0.1	18.2	3	2939.7
	Pm	705	0.3	2.5 +/-0.3	7.7	3	

Table 2: Deltamethrin concentration–response of different population of *Phytoseiulus macropilis* (Pm)

Species	N (total no of mites used)	LC_{50} (mg a.i./L)	Slope +/- SE	X^2	df	Resistance Ratio [a]
Pm1	705	0.3	2.5 +/-0.3	7.7	3	-
Pm2	325	281.9	1.9 +/- 0.2	4.5	3	854.1
Pm3	320	438.5	2.1 +/-0.3	4.4	3	1328.3
Pm4	448	1159.1	3.1 +/-0.4	4.1	3	3512.5

[a] =LC_{50} of the population under investigation/LC_{50} of the reference susceptible population (Pm)

CONCLUSION

The immature and adult stages of *N. californicus* were approximately 3,600 and 3,000-fold more tolerant to deltamethrin than those of *P. macropilis*. The LC_{50} values for *N. californicus* were 866.3 mg a.i./L for immature stage and 970.1 mg a.i./L for adults. The LC_{50} values for *P. macropilis* were 0.2 mg a.i./L for immature stage and 0.3 mg a.i./L for adults. However, high variability in the

susceptibility to this insecticide was detected among *P. macropilis* populations, with resistance ratios of up to 3,500-fold. LC_{50} values varied between 0.3 mg a.i./L and 1159.1 mg a.i./L.

Comment by the Notifier

The extended laboratory data on the most sensitive tested predatory mite population of *P. macropilis* indicate a similar susceptibility of this species (LC_{50} 0.3 mg a.i./L equivalent to 30 mg a.i./ha) as *T. pyri* (LR_{50} 16.5 mg a.i./ha) that has been tested for the regulatory data package. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.2/13; J. P. Jansen • T. Defrance • A. M. Warnier (2011)**
Title:	Side effects of flonicamid, and pymetrozine on five aphid natural enemy species
Source:	BioControl (2011) 56:759–770
DOI No.:	10.1007/s10526-011-9342-1
Document No.	M-462296-01-1
Guidelines:	IOBC standard sequential testing scheme for beneficial insects (Hassan et al. 1994).
GLP:	No

EXECUTIVE SUMMARY

The effects of deltamethrin (as toxic reference compound) on inert and natural substrates, on the rove beetle *Aleochara bilineata* (Gyll.), the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez), the ladybird *Adalia bipunctata* (L.) and the carabid beetle *Bembidion lampros* (Herbst), were assessed in the laboratory. Deltamethrin was found very toxic to all tested species. In all treatments (glass plate test and laboratory- or field-treated plants) mortality was significantly higher than in the control treatment.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis Micro WG
Active substance(s):	6.25 % w/w
Adjuvant / Surfactant:	None
Source of test item:	Not reported
Lot/Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported

2. Test solutions

Vehicle/solvent: the maximum recommended field rate was tested, diluted in 200 l spray mixture ha-1 for glass plate tests and 400 l ha^{-1} for tests on plants or sand

3. Test organism(s)

Species: -rove beetle *Aleochara bilineata* (Gyll.),

-parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez),

-ladybird *Adalia bipunctata* (L.)

-carabid beetle *Bembidion lampros* (Herbst)

Source of test species: *A. bipunctata* obtained by mass rearing in the laboratory, established in 1996 from adults sampled outside on ornamental bushes.
A. rhopalosiphi : obtained by mass rearing in the laboratory, established in 1994 from aphid mummies collected in winter wheat fields.
A. bilineata: provided by a commercial supplier ([redacted] The Netherlands) in the form of parasitised onion fly pupae.
B. lampros: caught in July and August in cereal field margins using pitfall traps and a small aspirator.

Age of test organisms at study initiation: *A. bipunctata*: 2–3 day old
A. rhopalosiphi: 0–48 h old
A. bilineata: 3–7 day old
B. lampros: not reported

Holding conditions prior to test: *A. bipunctata*: kept in Plexiglass cages and fed with an excess of aphids (a mixture of pea aphids, *Acyrtosiphon pisum* (Harris) reared on French beans (*Vicia fabae* L.) and green peach aphids, *Myzus persicae* (Sulzer) reared on sweet pepper (Capsicum annuum L.)) and honeybee-collected pollen.
A. rhopalosiphi: produced using the cereal grain aphid *Sitobion avenae* (F.) as the host aphid and barley seedlings (*Hordeum vulgare* L.) as the host plants
A. bilineata: plastic cages filled with wet sand and fed with frozen mosquito flies (Dissus fish food)
B. lampros: kept in large units on natural soils for 2–8 weeks at 20 ± 2 °C before being used for the tests. They were fed in excess with *Ephestia kuehniella* eggs (Nutrimac), aphids and onion fly pupae.

B. Study design and methods

1. Test procedure

Test system (study type): *A. bipunctata*: glass plates and plants
A. rhopalosiphi: glass plates and plants
A. bilineata pure quartz sand according to Grimm et al. 2000 and natural soil. Because of the effects observed on plants in the laboratory, an additional test was also performed with winter wheat (*Triticum aestivum* L.) plants treated in the field under conditions similar to conventional practice
B. lampros: pure quartz sand according to [redacted] et al. 2000 and natural soil

Duration of study: *A. bipunctata*: five consecutive 24-h periods or until pupation
A. rhopalosiphi: 48h for mortality and 10-12 d for fecundity
A. bilineata: 28-day exposure and 6–8 week emergence

	assessment *B. lampros:* mortality was checked on days 1,2, 4, 7, 11 and 14.
Test concentrations	5 g a.s./ha
Number of replicates:	*A. bipunctata* four replicates *A. rhopalosiphi* five replicates for glass plates; 6 replicates for treated plants *A. bilineata:* four replicates *B. lampros:* five replicates
Individuals per replicate:	*A. bipunctata* ten larvae *A. rhopalosiphi:* groups of ten (five males, five females) for mortality and 15 for fecundity *A. bilineata:* 20 rove beetles per treatment and 1,200 onion fly pupae added to each unit during the exposure period. *B. lampros:* six beetles
Test units (type and size):	*A. bipunctata:* glass disc (5 cm) surrounded by a plastic ring coated with Fluon GP1 to prevent the larvae from escaping *A. rhopalosiphi:* two treated glass plates (10 x 10 cm) held apart by an untreated metal frame (10 x 10 x 2 cm) *A. bilineata:* plastic box (17 x 12 x 6 cm) filled with 500 g sand wetted at 70% of its water-holding capacity. *B. lampros:* plastic box (17 x 12 x 6 cm) filled with 500 g sand.
Application / device / nozzles/water volume/ calibration:	For the glass plate tests, the spray solutions were applied to the substrate by use of a Laboratory Burgerjon spray tower (Burgerjon 1956) calibrated to deliver an application volume of 200 l ± 10% ha-1. For tests performed on sand and plants, the products were applied outdoors using a knapsack sprayer connected to a 2 m-wide ramp with four teejet flatfan nozzles (Teejet XR series, 110). The apparatus was calibrated to deliver an application volume of 400 l ± 10% ha-1.

2. Environmental conditions

Test medium:	Glass plates or treated plant leaves
Temperature / relative humidity:	20 ± 2 °C, 60–90% RH
Photoperiod:	16:8 L:D using a sodium lamp, except for *A. bilineata* (assessment in the dark)
Lighting	Light intensity was 1,000–2,000 lux for the 48 h of exposure and 7,000–10,000 lux during fertility assessment. These methods were based on existing guidelines for the registration of pesticides (Mead-Briggs et al. 2000).

3. Observations and measurements:

Analytical parameters measured:	none
Biological parameters measured:	Pre-imaginal mortality, Mortality, development time, viable eggs, reduction in fertility and incidence of parasitism

Measurement frequency: *Aleochara bilineata* test: 48 h

Statistical analyses: As required, treatment mortality was corrected for observed control mortality using Abott's formula (Abott 1925). The results of the tests were analysed using Statistical Minitab software. A one-way ANOVA test (LSD) for variance analysis was performed, followed by Tukey tests for multiple comparisons between treatments (P = 0.05).

RESULTS AND DISCUSSION

Validity criteria:

Validity criteria were defined according to guidelines.

Biological findings:

Mortality on *A. bipunctata* and *A. rhopalosiphi* tested on glass-plates was high for deltamethrin (100%) (table 1 and table 3). Mortality on *A. rhopalosiphi* on laboratory treated-plants was 31.7 %, and therefore significantly higher than in the control treatment. Significantly higher mortality was also observed for *A. rhopalosiphi* in the field treated plant treatment (table 3). Deltamethrin used as the toxic standard for *A. bilineata*, led to reduction in the incidence of parasitism of more than 50% compared with the control (table 2). Also for *B. lampros*, deltamethrin increased the mortality (43.4%) of the beetles significantly compared to the control (6.7%) (table 4).

Table 1: Toxicity of deltamethrin to larvae of the ladybird species *A. bipunctata* on glass plates in the laboratory

glass plate	Pre-imaginal mortality (mean ± SE)
Control	20.0 +/- 8.2 a
Deltamethrin	100.0 +/- 0.0 c

[a] Pre-imaginal mortality (%), development time to adult stage (days), and fertility of adult female obtained from the larvae exposed to the test products One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different (P = 0.05)

Table 2: Toxicity of deltamethrin to the rove beetle species *A. bilineata* on sand in the laboratory

	Parasitized onion fly pupae/unit (mean ± SE)	Incidence of parasitism	Parasitism reduction
Control	678.5 +/- 82.0 a	56.5	
Deltamethrin	70.3 +/- 43.8 c	5.9	89.8

[a] Mean number of parasitisized onion fly pupae, incidence of parasitism (%), and reduction in parasitism compared with control (%) One-way ANOVA (LSD) followed by Tukey test for multiple comparison. Results followed by different letters are significantly different (P = 0.05)

Table 3: Effects of deltamethrin on *A. rhopalosiphi* adults on glass plates and on plants

		48 h mortality (mean ± SE)	Mummies/female (mean ± SE) (number of females)	Reduction in fertility
glass plate	Control	0.0 +/- 0.0 a	22.8 +/-20.0 a	-
	Deltamethrin	100.0 +/- 0.0 c	not assessed	-
Treated plants	Control	1.7 +/- 4.1 a	21.9 +/- 21.3 a	-
	Deltamethrin	31.7 +/- 11.7 b	not assessed	-

Field-treated plants	control	6.0 +/- 8.0 a	17.3 +/- 9.4 a	-
	Deltamethrin	54.0 +/- 8.0	15.3 +/- 5.0 a	11.2

[a] Mortality after 48 h exposure (%), number of aphid mummies produced by females that survived the exposure (number of females assessed), and reduction of fertility compared with control (%) One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different (P = 0.05)

Table 4: Toxicity of deltamethrin to the ground beetle species *B. lampros* on sand in the laboratory

	Observed mortality (mean ± SE)	Corrected mortality
Control	6.7 +/- 9.1 a	
Deltamethrin	43.4 +/- 25.9 b	38.0

[a] Observed and corrected mortality (%) after 14 days of exposure One-way ANOVA followed by Tukey test for multiple comparison. Arcsin transformation for percentage. Results followed by different letters are significantly different (P = 0.05)

CONCLUSION

An application rate of 5 g a.s./ha of deltamethrin was found toxic to the rove beetle *Aleochara bilineata* (Gyll.), the parasitic wasp, *Aphidius rhopalosiphi* (DeStefani-Perez), the ladybird *Adalia bipunctata* (L.) and the carabid beetle *Bembidion lampros* (Herbst). In all treatments (glass plate test and laboratory- or field-treated plants), mortality was significantly higher than in the control treatment.

Comment by the Notifier

Compared to the available regulatory data package on NTAs are the data on *Aleochara bilineata*, *Aphidius rhopalosiphi*, *Adalia bipunctata,* and *Bembidion lampros* are in the expected effect range indicating no higher sensitivity of the tested species. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

*****

Report:	**KCP 10.3.2.2/14; Hautier, L.; Jansen, J.-P.; Mabon, N.; Schiffers, B. (2005)**
Title:	Selectivity lists of pesticides to beneficial arthropods for IPM programs in carrot - first results.
Source:	Commun. Agric. Appl. Biol. Sci., 70, 4, p. 547-557
DOI No: Document No:	- M-460887-01-1
Guidelines:	For aphids predators and parasites[34], for ground insects[35][36]

[34] Copin A. Latteur G., Deleu R., Mahaut T. & Schiffers B. (2001). Evaluation du risque de toxicité de pesticides vise -à-vis de trois auxiliaries (*Adalia bipunctata, Aphidius rhopalosiphi* et *Episyrphus baleteatus*) par le dosage chimique de résidus. Ministère des Classes moyennes et de l´Agricultue DG 6. 83 pages.

[35] [redacted] U., Dohmen P., Barrett K.L., Brown K., Kennedy P.J., [redacted], Römbke J., [redacted], Schmuck R., & Wilhelmy H. (2000). A method for testing effects of plant protection products on the carabid beetle Poecilus cupereus (Coleoptera, Carabidae) under laboratory and semi-field. In: Candolfi M.P. Bluemel S., Forster R., [redacted] M., Grimm C., Hassan S.A., [redacted] U., Mead-Briggs M.A., Reber B., Schmuck R. & Vogt H. Guidelines to evaluate side-effects of plant protection products to non-target arthropods. Bulletin OILB-SROP / IOBC-WRPS Bulletin. 158 pp

[36] Grimm C., Reber B., [redacted], Candolfi M.P., Drexler A., [redacted], Moreth L., Ufer A. & [redacted] (2000). A test for evaluating the chronic effects of plant protection products on the rove beetle Aleochara bilineata Gyll. (Col. Staphylinidae) under laboratory and extended labaratory conditions. In: Candolfi M.P., Bluemel S., Forster R., [redacted] M., Grimm C., Hassan S.A., [redacted] U., Mead.Briggs M.A .. Reber B.•

GLP:	no

EXECUTIVE SUMMARY

The aim of this research was to assess the toxicity of pesticides currently used in carrot crop towards natural enemies. 28 pesticides were tested. However, material and methods as well as results are summarized only for deltamethrin. The test concentrations were 10 g ai/ha which was applied with a pneumatic atomizer at 200±10% l/ha for glass and plants, and at 400±10% l/ha for sand and soil. Pesticides toxicity towards beneficial arthropods were assessed according to SETAC guidelines (Barrett et al., 1994), an original methodology developed by Copin et al. (200 1)[34] for aphids predators and parasites, and for ground insects from [redacted] et al (2000) and Grimm et al (2000)[36]. Five beneficial insects were selected for toxicity tests: adult *Aphidius rhopalosiphi*, larvae of *Adalia bipunctata*, larvae of *Episyrphus balteatus*, adult of *Bembidion lampros* and adult of *Aleochara bilineata*. The acute toxicity was assessed according to a sequential testing scheme. First step deltamethrin was tested on an inert substrate, glass or sand, according to the insect. Mortalities of aphids parasites and predators were assessed after 48 hours exposition or after 2 weeks for carabids and calculate corrected mortality (CM) were calculated (Abbot, 1925)[37]. For staphylinid, parasitism reduction (PR) was calculated after 4 weeks in comparison with control. If the prodct induced a corrected mortality or a parasitism reduction higher than 30% the toxicity was realised in semi-field controlled conditions on a natural substrate (horse bean for Syrphidae and Coccinellidae, barley for Aphidiidae, soil for Carabidae and Staphylinidae). In these conditions corrected mortality or parasitism reduction was calculated.
Furthermore, for each toxicity test, on glass or on plant, active ingredient on the substrate is measured by chemical analysis at the beginning and at the end of the test.
For *A. rhopalosiphi*, mortality on glass was 100% whereas the toxicity on plants indicated 75% morality. Tests with *A. bipunctata* showed 100% corrected mortality on glass and on plants. For *E. balteatus* mortality on glass was 75 and on plants 77%. On sand, *A. bilineata* showed 100% parasitism reduction. For *B. lampros*, deltamethrin were tested on sand and indicated a mortality of 72%.

MATERIAL AND METHODS

A. Material

1. Test material

Test item: Decis 2.5 EC
Active substance(s): Deltamethrin
Adjuvant / Surfactant: -
Source of test item: -
Lot/Batch number: -
Purity: -
Storage conditions: -

2. Test solutions

Vehicle/solvent: -
Source of vehicle/solvent: -
Concentration of vehicle/solvent: -

3. Test organism(s)

Schmuck R & Vogt H. Guidelines to evaluate side-effects of plant protection products to non-target arthropods. Bulletin OILB-SROP /IOBC-WRPS Bulletin. 158 pp

[37] Abbott S.W. (1925). A method of computing the effectiveness of insecticides. Journal of Economic Entomology, 18:265-267.

Species:	*A. rhopalosiphi*, *A. bipunctata*, *E. balteatus*, *A. bilineata*, *B. lampros*
Cultivar:	-
Source of test species:	-
Age of test organisms at study initiation / Crop growth stage at treatment:	adult *Aphidius rhopalosiphi*, larvae of *Adalia bipunctata*, larvae of *Episyrphus balteatus*, adult of *Bembidion lampros* and adult of *Aleochara bilineata*
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Acute toxicity
Duration of study:	48 h (Aphids parasites and pedatores); 2 weeks (carabids) and 4 weeks (staphylinid)
Treatments:	Decis and control
Test concentrations	10 g ai/ha
Number of replicates:	-
Individuals per replicate:	-
Test units (type and size):	According to Barrett et al. (1994), Copin et al. (2001)[34], [redacted] et al. (2000)[35] and Grimm et al. (2000)[36]
Application / device / nozzles:	-
Water volume:	-
Calibration of sprayer:	-

2. Environmental conditions

Test medium:	Glass, Sand, horse bean, barley, soil
Temperature / relative humidity:	According to Barrett et al. (1994), Copin et al. (2001)[34], [redacted] et al. (2000)[35] and Grimm et al. (2000)[36]
Photoperiod:	According to Barrett et al. (1994), Copin et al. (2001)[34], [redacted] et al. (2000)[35] and Grimm et al. (2000)[36]
Lighting:	According to Barrett et al. (1994), Copin et al. (2001)[34], [redacted] et al. (2000)[35] and Grimm et al. (2000)[36]
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements:

Analytical parameters measured:	-
Biological parameters measured:	Mortality and parasitism reduction (PR)
Measurement frequency:	Only at test end
Statistical analyses:	Abott (1925)[37]

RESULTS

Validity criteria:

No validity criteria were stated

Biological findings:

Table 1: Results of toxicity tests, corrected mortality (CM) or parasitism reduction (PR) (%), A: results on inert substrate (glass or sand): B; results in semi-controlled conditions (plants or soil); §: not yet completely tested.

Active ingredient	Formulation	a.i. concentration (%)	g a.i./ha	A. rhopalosiphi		A. bipunctata		W. balteatus		A. bilineata		B. lampros	
				A.	B	A	B	A	B	A	B	A	B
Deltamethrin	Decis 2.5 EC	2.5	10	100	75	100	100	7	77	100	-	72	-

For *A. rhopalosiphi*, mortality on glass was 100% whereas the toxicity on plants indicated 75% morality. Tests with *A. bipunctata* showed 100% corrected mortality on glass and on plants. For E. balteatus mortality on glass was 75 and on plants 77%. On sand, *A. bilineata* showed 100% parasitism reduction. For *B. lampros*, deltamethrin were tested on sand and indicated a mortality of 72%.

Comment by the Notifier

The presented data confirm the known acute toxicity of deltamethrin to insects at an application rate of 10 g a.s./ha under laboratory and extended laboratory conditions. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

CP 10.3.2.3 Semi-field studies with non-target arthropods

No semi-field studies have been conducted since field tests are available.

CP 10.3.2.4 Field studies with non-target arthropods

Report:	**KCP 10.3.2.4/01, [redacted], [redacted]; 2012**
Title:	A field study to assess the effects of Deltamethrin EW 15 (g/L) on the non-target, surface- and plant-dwelling arthropod fauna of a grassland habitat (off-crop) in The Netherlands during spring/summer
Document No.:	M-430876-03-2 (EBDAL045)
Guidelines:	IOBC (Hassan, 1992), Anonymous (1992), Brown (1998), IOBC, BART and EPPO Joint Initiative (Candolfi et al., 2000, 2001), De Jong et al. (2010)
GLP:	yes

Objective

Deltamethrin EW 15 is an insecticide. This field study was designed to assess the potential adverse effects on Non-Target Arthropods (NTA) in off-crop habitats that might occur at various distances from a treated area for current and future use patterns of the test item. By analogy to regulatory studies in e.g. aquatic environments the study was set up to enable an assessment of community- and

population level ecotoxicological standards, in particular the NOER (No Observed Effect Rate), the NOEAER and the LOEAER (No and Lowest Observed Ecologically Adverse Effect Rate, respectively).
This study was performed in a true off-crop habitat, i.e. a grassland habitat with little agricultural input in The Netherlands. The site was situated in an agricultural area. This approach had the advantage that the observed response would pertain to a more representative off-crop NTA community, i.e. a community not previously under selection in an agricultural regime. For this reason the study outcome will represent a realistic worst case situation, irrespective of the intended product use.
The study was performed as a NOER-type study ([redacted] 2007; De Jong *et al.* 2010).

To enable a refined assessment of NOEAER/LOEAER sampling was continued until 8 weeks post-application.

Methods

Deltamethrin EW 15 was applied once to a grassland meadow on 1 July 2011 at nominal rates of 0.1, 0.23, 0.6, 1.3 and 3.0 g a.s./ha, equivalent to typical drift values for different use patterns of the test item. Average application rates per treatment deviated 7% or less from intended rates. A water control treatment and a toxic reference treatment (lambda-cyhalothrin at a rate of 0.4 L product/ha) were run in parallel. Nominal application volumes were 200 L/ha.
The soil-surface- and plant-dwelling arthropod communities were monitored shortly before, one, two, four and eight weeks after application. A broad spectrum of arthropods was sampled with different sampling methods, viz. pitfall trapping, Berlese-Tullgren extraction from weed samples and suction sampling.
The trial had a randomized complete block design with 4 replicates/treatment. Each block had seven treatment plots of 24 x 24 m. To minimize interference among plots, the trial was laid out in a checkerboard design.
The effects of Deltamethrin EW 15 were expressed in terms of population and community changes relative to the water control. The No Observed Effect Rate (NOER) was defined at the community level and at the population level as the rate at which adverse responses were not significantly different from the water control at any time point. The No Observed Ecologically Adverse Effect Rate (NOEAER) was defined at the community level and at the population level as the rate at which statistically significant adverse responses were observed, but recovery was demonstrated within two months after applications. By analogy the LOEAER (for community and population responses) was defined as the lowest test rate at which adverse effects were significantly different from the water control without recovery occurring.
Statistical significances were in principal considered at an alpha level of 5%. Statistical significances at an alpha level of 10% were also indicated as additional information to evaluate potential trends.

Findings

Biological system:
The arthropod community sampled in this study was diverse and typical for grassland vegetation, and representative for an off-crop non-target arthropod community. The timing of the experiment was such that a high number of abundant taxa were present during the sampling period. In addition timing coincided with typical use patterns for the test item. The entire dataset was appropriate for community analyses using ordination techniques. In addition, a total of 62 taxa were sufficiently abundant to be subjected to population level evaluations. A number of evaluations were performed at the family level,

but several taxa occurred at sufficiently high numbers to allow for an evaluation at genus or species level.
The taxonomical analysis was performed in great detail. Despite the restrictions caused by the inevitable categorization of specimens at different taxonomic levels, it was felt that the number of taxa together with the choice of taxonomic level used for analysis did provide a sufficiently detailed and valid ecological analysis.

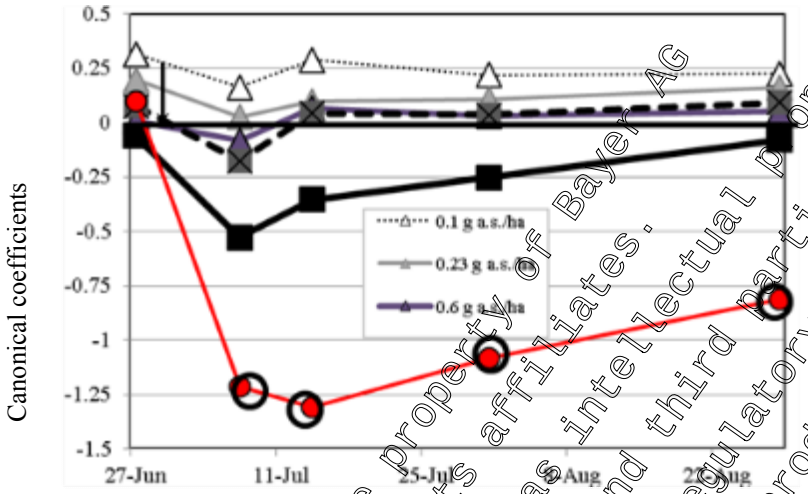
Sampling strategy
The entire arthropod community occurring in the off-crop habitat was monitored using pitfall-, weed/Berlese and suction sampling techniques. There was some overlap of taxa sampled with the different trapping techniques. Because of taxonomic differences (different species in the same higher level taxon), biological differences (e.g. life stages with different susceptibility in different traps) or behavioural differences (e.g. different exposure in different sub-habitats sampled), taxa sampled with different techniques were considered different taxa for the overall community analyses (based on a pooled dataset with all sampling methods included).

Test performance (insecticidal reference treatment)
At the family level there were no fundamental differences in the composition of the off-crop arthropod fauna in comparison to agricultural sites. The number of taxa occurring at sufficiently high numbers to allow for a population level analysis was higher than the number of taxa usually evaluated in studies performed in commercial agricultural settings.
By using three different collecting methods (weed/Berlese sampling, pitfall, suction) the arthropod community occurring in grasslands was comprehensively sampled (ground- and plant dwelling arthropods).
Application of the insecticidal toxic reference item lambda-cyhalothrin resulted in clear responses at both the arthropod community level and the population level. This was true for taxa and communities collected with all three sample types.
The overall PRC obtained from community analyses of all sample types combined was statistically significant for the toxic reference treatment. On individual sampling moments the response was statistically significant in comparison to the control on all post application moments. At the population level many taxa appeared adversely and statistically significantly affected. Indirect effects were also observed: numbers of some Collembola taxa were significantly increased compared to the control, probably due to reduced predation by spiders which were adversely affected by the toxic reference item.
For several taxa no recovery was seen in the toxic reference treatment within the two-month sampling period, indicating that the experimental period and plot size chosen were adequate to demonstrate persistent treatment related effects. Abbott values in the toxic reference treatment were above 50% for approximately half of all taxa examined during the entire post-treatment period. Consequently validity criteria according to De Jong *et al.* (2010) were met.
It is concluded that the test method presented in this study accurately examined potential risks for NTA fauna in true and representative off-crop habitats under a realistic worst-case test scenario.

Results Deltamethrin EW 15
Treatment with the insecticide Deltamethrin EW 15 in an off-field grassland habitat in The Netherlands led to a statistically significant adverse effect on prevailing arthropod communities only for the highest test rate of 3 g a.s./ha, on only one sampling occasion one week after application, and only in the suction- and the pitfall dataset. Visual inspection of the PRC graph confirmed that at the

community level a moderate and transient treatment related response could be observed. With all sampling methods analyzed together differences compared to the control were not statistically significant.

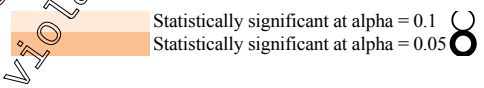
Summary community level effects

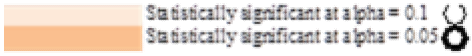


sample	taxon	species scores
P	Poduromorpha	4.516
P	Alticinae	2.1644
S	Chloropidae	1.9324
P	Lathridiidae	1.9215
P	Amara	1.8324
S	Lepidoptera (adults)	1.7717
P	Phalangida (adult)	1.7715
P	Coccinellidae (adults)	1.769
S	Miridae (juveniles)	1.7242
P	Curculionidae	1.7072
P	Aleocharinae	1.4922
S	Cecidomyiidae	1.4748
S	Mymaridae	1.4395
S	Cynipoidea	1.402
P	Entomobryidae	1.3801
W	Phoridae (female)	1.3776
S	Chalcidoidea (juveniles)	1.3638
S	Eurytomidae	1.1952
P	Agonum sp.	1.1952
S	Pachygnatha (juvenile)	1.1873
P	Pardosa (adult)	1.1454
P	Carabidae (juveniles)	1.1366
S	Scelionidae	-1.2576
W	Pyrgotoidea	-1.3201

Principal Response Curve (first ordination axis)

Test- and toxic reference items were analyzed separately but for comparison plotted in one graph. PRC analyses comprised data from weed (W)-, pitfall (P)- and suction (S) samples. Encircled data points are statistically significant (Monte-Carlo Permutation test, alpha = 0.05). The 25 largest species scores of the test item treatments are presented (i.e. these species had the largest influence on the shape of the PRC curves). The arrow indicates the application day.





Treatments in analysis	% Variance accounted for by		% Variance explained by treatment captured by			
	time	treatment	ax1	ax2	P-value ax1	P-value ax2
Test item rates	30.5	12.3	17.7	10.4	0.928	1.000
Reference	26.4	23.4	73.3	13.4	0.027	0.054

P-values at individual sampling moments (Monte Carlo Permutation Test)

All data	sample	0.1 g a.s./ha	0.23 g a.s./ha	0.6 g a.s./ha	1.3 g a.s./ha	3 g a.s./ha	Reference
28-Jun-11	1	0.366	0.942	0.977	0.900	0.748	0.862
08-Jul-11	2	0.910	0.887	0.910	0.509	0.131	0.029
15-Jul-11	3	0.452	0.869	0.813	0.660	0.495	0.035
01-Aug-11	4	0.761	1.000	0.963	0.919	0.638	0.032
29-Aug-11	5	0.818	1.000	1.000	0.917	1.000	0.027
Weed data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
28-Jun-11	1	0.321	0.790	0.956	0.703	0.392	0.971
08-Jul-11	2	0.937	0.841	0.975	0.732	0.886	0.235
15-Jul-11	3	0.838	0.910	0.858	0.970	0.758	0.035
01-Aug-11	4	1.000	0.946	0.827	0.608	0.815	0.023
30-Aug-11	5	0.829	0.823	0.882	1.000	0.997	0.028
Pitfall data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
01-Jul-11	1	0.276	0.947	0.895	0.930	0.719	0.719
08-Jul-11	2	0.742	0.866	0.828	0.223	0.043	0.028
15-Jul-11	3	0.234	0.302	0.571	0.304	0.176	0.031
05-Aug-11	4	0.634	1.000	1.000	0.892	0.664	0.029
26-Aug-11	5	0.583	0.864	0.974	0.812	0.793	1.000
Suction data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
28-Jun-11	1	0.662	0.862	0.887	0.762	0.941	0.836
08-Jul-11	2	0.632	0.885	0.830	0.617	0.050	0.031
15-Jul-11	3	0.446	0.946	0.909	1.000	0.608	0.037
01-Aug-11	4	0.389	1.000	0.884	0.920	0.811	0.032
29-Aug-11	5	0.496	1.000	1.000	0.844	1.000	0.045

Treatments in analysis	% Variance accounted for by		% Variance explained by treatment captured by			
	time	treatment	ax1	ax2	P-value ax1	P-value ax2
Test item rates	30.5	12.3	17.7	10.4	0.928	1.000
Reference	26.4	23.4	73.3	13.4	0.027	0.054

P-values at individual sampling moments (Monte Carlo Permutation Test)

All data	sample	0.1 g a.s./ha	0.23 g a.s./ha	0.6 g a.s./ha	1.3 g a.s./ha	3 g a.s./ha	Reference
28-Jun-11	1	0.366	0.942	0.977	0.900	0.743	0.862
08-Jul-11	2	0.910	0.887	0.910	0.509	0.131	0.029
15-Jul-11	3	0.452	0.869	0.813	0.660	0.495	0.027
01-Aug-11	4	0.761	1.000	0.963	0.919	0.638	0.032
29-Aug-11	5	0.818	1.000	1.000	0.917	1.000	0.027
Weed data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
28-Jun-11	1	0.321	0.790	0.856	0.703	0.392	0.975
08-Jul-11	2	0.937	0.841	0.975	0.732	0.886	0.235
15-Jul-11	3	0.838	0.910	0.858	0.470	0.756	0.035
01-Aug-11	4	1.000	0.946	0.837	0.608	0.975	0.023
30-Aug-11	5	0.829	0.823	0.882	1.000	0.797	0.026
Pitfall data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
01-Jul-11	1	0.276	0.947	0.895	0.930	0.719	0.719
08-Jul-11	2	0.742	0.866	0.825	0.223	0.045	0.028
15-Jul-11	3	0.234	0.302	0.571	0.304	0.176	0.031
05-Aug-11	4	0.634	1.000	1.000	0.894	0.664	0.029
26-Aug-11	5	0.583	0.864	0.971	0.812	0.793	0.097
Suction data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
28-Jun-11	1	0.362	0.862	0.857	0.762	0.941	0.830
08-Jul-11	2	0.632	0.869	0.830	0.617	0.050	0.031
15-Jul-11	3	0.446	0.906	0.909	1.000	0.606	0.037
01-Aug-11	4	0.389	1.000	0.884	0.920	0.441	0.032
29-Aug-11	5	0.406	1.000	1.000	0.841	1.000	0.045

Summary population level effects

Effect classification:		Effect class:
one occasion	Clear adverse treatment related effect but observed only on one occasion	2
< 2 months (a)	Adverse effect no longer statistically significant on the last two sampling moments	3a
< 2 months (b)	Adverse effect no longer statistically significant on the last sampling moment	3b
> 2 months	No recovery from adverse effect within the study period (= 2 months)	8

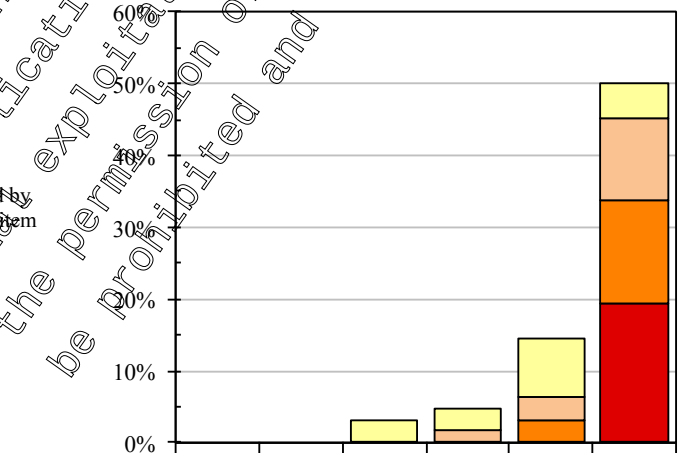
Based on 10% significance level and visual consideration of trends	2
Based on 5% significance level and visual consideration of trends	3

Order	Taxon	method	0.1 g	0.23 g	0.6 g	1.3 g	3 g	Ref
ACARI	Phytoseiidae (female)	W						8
ACARI	Gamasida other (female)	W						8
ACARI	Gamasida Larvae	W						8
ACARI	Gamasida Nymph and male	W						8
COLEOPTERA	Alticinae	P				2	2	3a
COLEOPTERA	Curculionidae	P						
COLEOPTERA	Coccinellidae (adults)	P					2	3b
COLEOPTERA	Lathridiidae	P						8
ARACHNIDA	Phalangida	P						8
ARANEAE	Pardosa (juvenile)	P						8
ARANEAE	Pardosa (adult)	P			2	3a	3a	3b
ARANEAE	Trochosa (adult)	P						3a
ARANEAE	Pachygnatha	P						3b
ARANEAE	Erigoninae	P						3b
THYSANOPTERA	Thysanoptera	S					2	8
HOMOPTERA	Aphidoidea	S						3b
HOMOPTERA	Cicadellidae (juveniles)	S					3b	8
HOMOPTERA	Cicadellidae (adults)	S					3b	8
HOMOPTERA	Delphacidae	S						2
DIPTERA	Cecidomyiidae	S			2	2	2	3b
DIPTERA	Lonchopteridae	S						3b
DIPTERA	Phoridae	S						2
DIPTERA	Agromyzidae	S					2	2
DIPTERA	Chloropidae	S					3a	3a
DIPTERA	Sphaeroceridae	S						3a
HYMENOPTERA	Braconidae	S						3a
HYMENOPTERA	Eulophidae	S						3a
HYMENOPTERA	Mymaridae	S						8
HYMENOPTERA	Cynipoidea	S						8
COLEOPTERA	Alticinae	S						3a
ARANEAE	Pachygnatha (juvenile)	S						3b
ARANEAE	Erigoninae	S						3b

Total number of taxa evaluated: 62

W=weed samples/Berlese extraction
P=pitfall
S-suction

Only taxa that were adversely affected by the test item or by the toxic reference item are shown.



	0.1 g	0.23 g	0.6 g	0.0%	0.0%	Ref
one occasion	0.0%	0.0%	3.2%	3.2%	8.1%	4.8%
< 2 months (a)	0.0%	0.0%	0.0%	1.6%	3.2%	11.3%
< 2 months (b)	0.0%	0.0%	0.0%	0.0%	3.2%	14.5%
> 2 months	0.0%	0.0%	0.0%	0.0%	0.0%	19.4%

According to De Jong *et al.* (2010), recovery should be demonstrated on two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling moments) and class 3b (no longer statistically significant on the last sampling moment).

At the population level nine taxa showed statistically significant adverse response patterns that were considered related to the test item treatment (based on magnitude and duration in relation to dose and timing). These were two Coleoptera taxa (Alticinae, Chrysomelidae: 1.3 g and 3 g a.s./ha, and adult Coccinellidae: 3 g a.s./ha), one spider taxon (adult *Pardosa*, Lycosidae; 0.6 g, 1.3 g and 3 g a.s./ha), Thysanoptera (3 g a.s./ha), juvenile and adult Cicadellidae (Homoptera; 3 g a.s./ha), and three dipteran taxa (Cecidomyiidae; 0.6 g, 1.3 g and 3 g a.s./ha, Agromyzidae and Chloropidae; 3 g a.s./ha). For few other taxa reductions compared to the control occurred incidentally, but no consistent trend in time or relation to the dose rate was found.

Summary table effect classifications Deltamethrin EW 15 rates

Community level effects (PRC/Monte-Carlo; 5% alpha level)	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**
			Effect class		
Weed/Berlese dataset	1	1	1	1	1
Pitfall dataset	1	1	1	1	1
Suction dataset	1	1	1	1	2
Conclusion				**Community NOER**	**Community NOEAER**

Population level effects (Mann-Whitney U test; 5% alpha level)	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**
			Effect class		
Alticinae (Chrysomelidae, Coleoptera)	1	1	1	2	2
adult Coccinellidae (Coleoptera)	1	1	1	1	2
adult *Pardosa* (Lycosidae, Araneae)	1	1	2	3a	3a
Thysanoptera	1	1	1	1	2
juvenile Cicadellidae (Homoptera)	1	1	1	1	3b
adult Cicadellidae (Homoptera)	1	1	1	1	3b
Cecidomyiidae (Nematocera, Diptera)	1	1	2	2	2
Agromyzidae (Acalyptrata, Diptera)	1	1	1	1	2
Chloropidae (Acalyptrata, Diptera)	1	1	1	1	3a
Conclusion		**Population NOER**			**Population NOEAER**

NOER: No Observed Effect Rate (no statistically significant differences compared to control)

NOEAER: No Observed Ecologically Adverse Effect Rate (at least 1 taxon with effect class 2 or 3, i.e. clear response to treatment but with recovery within 2 months after application)

Effect classification:		Effect class:
no effect	No consistent treatment related statistically significant differences compared to the control	1
one occasion	Clear adverse treatment related effect but observed only on one occasion	2
< 2 months (a)	Adverse effect no longer statistically significant on the last two sampling moments	3a
< 2 months (b)	Adverse effect no longer statistically significant on the last sampling moment	3b
> 2 months	No recovery from adverse effect within the study period (= 2 months)	8

Conclusions

It is concluded that Deltamethrin EW 15 applied at a rate of 3 g a.s./ha in an off-crop grassland in the Netherlands is the community NOEAER (No Observed Ecologically Adverse Effect Rate). No statistically significant adverse effects were found in the 1.3 g a.s./ha rate. This rate is classified as the community NOER (No Observed Effect Rate).

At the population level nine taxa were considered adversely affected by treatment with Deltamethrin EW 15 applied at a rate of 3 g a.s./ha, three taxa by treatment with 1.3 g a.s./ha and two taxa by treatment with 0.6 g a.s./ha. These taxa all recovered within two to eight weeks after application.

Deltamethrin EW 15 applied at 3 g a.s./ha is therefore classified as population NOEAER and 0.23 g a.s./ha as the population NOER.

*****

Report:	**KCP 10.3.2.4/02, [redacted], [redacted]; 2012**
Title:	A field study to assess the effects of Deltamethrin EW 15 (g/L) on the non-target, surface- and plant-dwelling arthropod fauna of a grassland habitat (off-crop) in SW France during spring/summer
Document No:	M-430827-01-1 (EBDAL069)
Guidelines:	IOBC (Hassan, 1992), Anonymous (1992), Brown (1998), IOBC, BART and EPPO Joint Initiative (Candolfi et al., 2000, 2001), De Jong et al. (2010)
GLP:	yes

Objective

Deltamethrin EW 15 is an insecticide. This field study was designed to assess the potential adverse effects on Non-Target Arthropods (NTA) in off-crop habitats that might occur at various distances from a treated area for current and future use patterns of the test item. By analogy to regulatory studies in e.g. aquatic environments the study was set up to enable an assessment of community- and population level ecotoxicological endpoints, in particular the NOER (No Observed Effect Rate), the NOEAER and the LOEAER (No and Lowest Observed Ecologically Adverse Effect Rate, respectively).

This study was performed in a true off-crop habitat, i.e. a grassland habitat with little agricultural input in SW France. The site was situated in an agricultural area. This approach had the advantage that the observed response would pertain to a more representative off-crop NTA community, i.e. a community not previously under selection in an agricultural regime. For this reason the study outcome represents a realistic worst case situation, irrespective of the intended product use.

The study was performed as a NOER-type study ([redacted], 2007; De Jong *et al*, 2010.

To enable a refined assessment of NOEAER/LOEAER sampling was continued until 8 weeks post-application.

Methods

Deltamethrin EW 15 was applied once to a grassland meadow on 2 June 2011 at nominal rates of 0.1, 0.23, 0.6, 1.3 and 3.0 g a.s./ha, equivalent to typical drift values for different use patterns of the test item. Average application rates per treatment deviated 2.6% or less from intended rates. A water control treatment and a toxic reference treatment (lambda-cyhalothrin at a rate of 0.4 L product/ha) were run in parallel. Nominal application volumes were 200 L/ha.

The soil-surface- and plant-dwelling arthropod communities were monitored shortly before, one, two, four and eight weeks after application. A broad spectrum of arthropods was sampled with different

sampling methods, viz. pitfall trapping, Berlese-Tullgren extraction from weed samples and suction sampling.
The trial had a randomized complete block design with 4 replicates/treatment. Each block had seven treatment plots of 24 × 24 m. To minimize interference among plots, the trial was laid out in a checkerboard design.
The effects of Deltamethrin EW 15 were expressed in terms of population and community changes relative to the water control. The No Observed Effect Rate (NOER) was defined at the community level and at the population level as the rate at which adverse responses were not significantly different from the water control at any time point. The No Observed Ecologically Adverse Effect Rate (NOEAER) was defined at the community level and at the population level as the rate at which statistically significant adverse responses were observed, but recovery was demonstrated within two months after applications. By analogy the LOEAER (for community and population responses) was defined as the lowest test rate at which adverse effects were significantly different from the water control without recovery occurring.
Statistical significances were in principal considered at an alpha level of 5%. Statistical significances at an alpha level of 10% were also indicated as additional information to evaluate potential trends.

Findings
Biological system
The arthropod community sampled in this study was diverse and typical for grassland vegetation, and representative for an off-crop non-target arthropod community. The timing of the experiment was such that a high number of abundant taxa were present during the sampling period. In addition timing coincided with typical use patterns for the test item. The entire dataset was appropriate for community analyses using ordination techniques. In addition, a total of 80 taxa were sufficiently abundant to be subjected to population level evaluations. A number of evaluations were performed at the family level, but several taxa occurred at sufficiently high numbers to allow for an evaluation at genus or species level.
The taxonomical analysis was performed in great detail. Despite the restrictions caused by the inevitable categorization of specimens at different taxonomic levels, it was felt that the number of taxa together with the choice of taxonomic level used for analysis did provide a sufficiently detailed and valid ecological analysis.

Sampling strategy
The entire arthropod community occurring in the off-crop habitat was monitored using pitfall-, weed/Berlese and suction sampling techniques. There was some overlap of taxa sampled with the different trapping techniques. Because of taxonomic differences (different species in the same higher level taxon), biological differences (e.g. life stages with different susceptibility in different traps) or behavioural differences (e.g. different exposure in different sub-habitats sampled), taxa sampled with different techniques were considered different taxa for the overall community analyses (based on a pooled dataset with all sampling methods included).

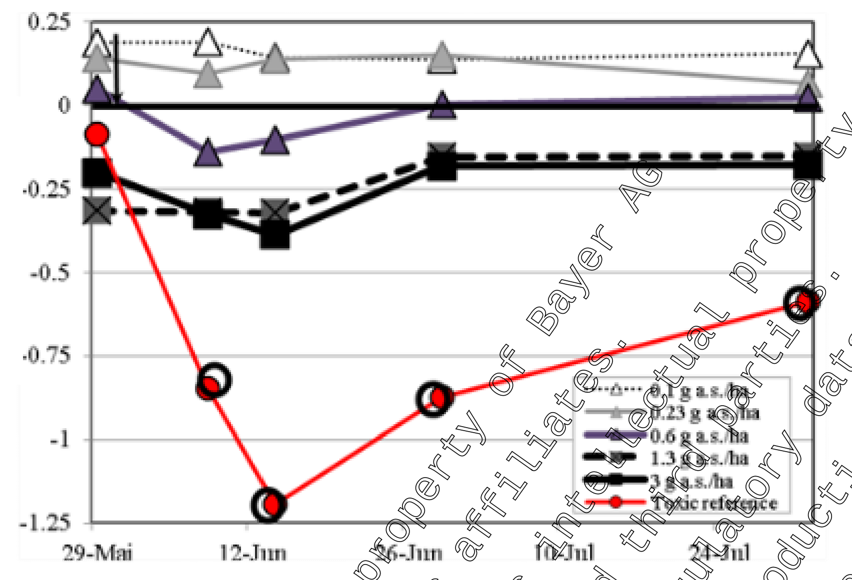
Test performance (insecticidal reference treatment)
At the family level there were no fundamental differences in the composition of the off-crop arthropod fauna in comparison to agricultural sites. The number of taxa occurring at sufficiently high numbers to allow for a population level analysis was higher than the number of taxa usually evaluated in studies performed in commercial agricultural settings.

By using three different collecting methods (weed/Berlese sampling, pitfall, suction) the arthropod community occurring in grasslands was comprehensively sampled (ground- and plant dwelling arthropods).
Application of the insecticidal toxic reference lambda-cyhalothrin resulted in clear responses at both the arthropod community level and the population level. This was true for taxa and communities collected with all three sample types.
The overall PRC obtained from community analyses of all sample types combined was statistically significant for the toxic reference treatment. On individual sampling moments the response was statistically significant in comparison to the control on all post application moments. At the population level many taxa appeared adversely and statistically significantly affected. Indirect effects were also observed: numbers of the collembolan taxa Entomobryidae, Sminthuridae and Symphypleona were significantly increased compared to the control, probably due to reduced predation by spiders which were adversely affected by the toxic reference.
For several taxa no recovery was seen in the toxic reference treatment within the two-month sampling period, indicating that the experimental period and plot size chosen were adequate to demonstrate persistent treatment related effects. Abbott values in the toxic reference treatment were above 50% for at least 40% of all taxa examined one and two weeks after application. Consequently validity criteria according to De Jong *et al.* (2010) were met.
It is concluded that the test method presented in this study accurately examined potential risks for NTA fauna in true and representative off-crop habitats under a realistic worst-case test scenario.

Results

Treatment with the insecticide Deltamethrin EW 15 in an off-field grassland habitat in South-West France did not lead to statistically significant effects on prevailing arthropod communities for any of the rates tested up to 3 g a.s./ha. Visual inspection of the PRC graph confirmed that at the community level no treatment related response could be observed.

Summary community level effects



sample	taxon	species scores
P	Alticinae	3.07[illegible]
W	Tanyderidae	[illegible].5436
S	Opomyzidae	[illegible].5335
W	Trichobates incisellus	2.4773
S	Eurytomidae	2.20[illegible]
W	Erythraeidae	2.0874
P	Ichneumonidae	2.0537
S	Pteromalidae	1.932
P	Phalangida (adult)	1.8874
W	Phaenopelopidae	1.8712
S	Apion spp.	1.8411
S	Eulophidae	1.8161
P	Elateridae	1.8017
S	Thysanoptera (adults)	1.6612
P	Anthicidae	1.6123
S	Miridae (adults)	1.563
P	Pachygnatha (adult)	1.5374
S	Ichneumonidae	1.5347
P	Amara	1.4938
P	Carabidae (other adults)	1.4658
S	Diapriidae	1.4326
W	Scheloribates laevigatus	1.4277
S	Cercopida	1.4151
S	Cercopidae (adults)	1.3848

Principle Response Curve (first ordination axis)

Test- and toxic references were analyzed separately but for comparison plotted in one graph. PRC analyses comprised data from weed (W)-, pitfall (P)- and suction (S) samples. Encircled data points are statistically significant (Monte-Carlo Permutation test, alpha = 0.05). The 25 largest species scores of the test item treatments are presented (i.e. these species had the largest influence on the shape of the PRC curves).

Statistically significant at alpha = 0.05 ○

treatments included in analysis	% Variance accounted for by: time	% Variance accounted for by: treatment	% Variance explained by treatment captured by: ax1	% Variance explained by treatment captured by: ax2	P-value ax1	P-value ax2
all	38.7	16.2	31.8	9.3	0.001	0.803
test item rates	41.1	11.8	18.1	9.3	0.621	0.998
0.1 g a.s./ha	47.2	6.5	44.7	17.8	0.645	0.971
0.23 g a.s./ha	44.4	5.6	38.2	20.6	0.943	0.932
0.6 g a.s./ha	44.5	6.2	38	20.3	0.893	0.956
1.3 g a.s./ha	47.2	6.8	40.1	22.5	0.515	0.844
3 g a.s./ha	43.6	7.2	43.3	19.8	0.606	0.648
Reference	40.9	16.5	69.8	10.7	0.033	0.373

P-values at individual sampling moments (Monte Carlo Permutation Test)

All data	sample	0.1 g a.s./ha	0.23 g a.s./ha	0.6 g a.s./ha	1.3 g a.s./ha	3 g a.s./ha	Reference
30-May-11	1	0.760	0.943	0.914	0.401	0.804	0.929
09-Jun-11	2	0.548	0.790	0.742	0.318	0.498	0.021
15-Jun-11	3	0.669	0.944	0.864	0.642	0.471	0.029
30-Jun-11	4	0.789	0.828	0.837	0.914	0.942	0.027
02-Aug-11	5	0.849	0.943	0.908	0.869	0.643	0.035
Weed data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
26-May-11	1	0.389	0.892	0.785	0.466	0.779	0.791
09-Jun-11	2	0.846	0.440	0.426	0.142	0.160	0.037
16-Jun-11	3	0.318	0.706	0.524	0.820	0.368	0.044
30-Jun-11	4	0.608	0.749	0.896	0.497	0.915	0.048
30-Jul-11	5	0.434	1.000	0.850	0.745	0.648	0.354
Pitfall data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
01-Jun-11	1	0.471	0.612	0.621	0.343	0.730	0.526
09-Jun-11	2	0.633	0.973	1.000	0.655	0.638	0.034
16-Jun-11	3	1.000	1.000	0.977	0.325	0.378	0.024
30-Jun-11	4	0.759	0.872	0.465	0.839	0.898	0.026
02-Aug-11	5	0.828	0.941	0.672	0.509	0.136	0.410
Suction data	**sample**	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**	**Reference**
30-May-11	1	0.851	1.000	0.878	0.525	0.664	0.942
09-Jun-11	2	0.405	0.674	0.370	0.284	0.598	0.027
15-Jun-11	3	0.692	0.829	0.899	0.655	0.629	0.019
30-Jun-11	4	0.891	0.671	0.863	0.800	0.815	0.028
02-Aug-11	5	0.819	0.672	0.693	0.903	0.714	0.035

Summary population level effects

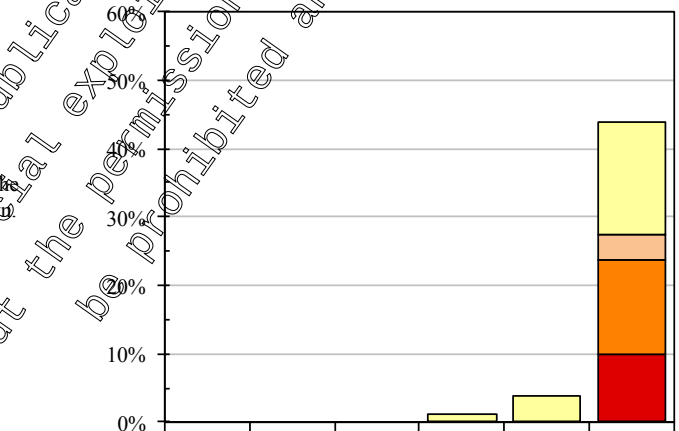
Effect classification:		
one occasion	Clear adverse treatment related effect but observed only on one occasion	2
< 2 months (a)	Adverse effect no longer statistically significant on the last two sampling moments	3a
< 2 months (b)	Adverse effect no longer statistically significant on the last sampling moment	3b
> 2 months	No recovery from adverse effect within the study period (= 2 months)	8

							Based on 10% significance level and visual consideration of trends	3
							Based on 5% significance level and visual consideration of trends	2
Order	Taxon	method	0.1 g	0.23 g	0.6 g	1.3 g	3 g	Ref
ACARI	Phytoseiidae (female)	W						3b
ACARI	Gamasida Larvae	W						8
ACARI	Gamasida Nymph and male	W						8
ACARI	Zygoribatula	W						2
COLLEMBOLA	Poduromorpha	P					2	
COLEOPTERA	Coccinellidae (adults)	P					2	2
COLEOPTERA	Corylophidae	P						2
COLEOPTERA	Lathridiidae	P						2
COLEOPTERA	Histeridae	P						2
COLEOPTERA	Aleocharinae	P						3a
ARACHNIDA	Phalangida	P						2
ARANEAE	Zelotes	P						2
ARANEAE	Pardosa (adult)	P						3a
ARANEAE	Lycosidae others	P						2
ARANEAE	Pachygnatha	P						8
ARANEAE	Linyphiidae	P						3b
THYSANOPTERA	Thysanoptera (juveniles)	S						(8)
THYSANOPTERA	Thysanoptera (adults)	S				2	2	8
HOMOPTERA	Aphidoidea	S						8
HOMOPTERA	Cicadellidae (juveniles)	S						3b
HOMOPTERA	Cicadellidae (adults)	S						3b
HETEROPTERA	Miridae	S						3b
LEPIDOPTERA	Lepidoptera (juveniles)	S						2
DIPTERA	Cecidomyiidae	S						8
DIPTERA	Chloropidae	S						2
HYMENOPTERA	Formicidae	S						3b
HYMENOPTERA	Braconidae	S						3b
HYMENOPTERA	Mymaridae	S						2
HYMENOPTERA	Scelionidae	S						2
COLEOPTERA	Coccinellini (juveniles)	S						3b
ARANEAE	Xysticus (juvenile)	S						2
ARANEAE	Salticidae (juvenile)	S						3b
ARANEAE	Araneidae (juvenile)	S						3b
ARANEAE	Meioneta	S						3b
ARANEAE	Other Linyphiidae	S						8

Total number of taxa evaluated: 80

W=weed samples/Berlese extraction
P=pitfall
S-suction

Only taxa that were adversely affected by the test item or by the toxic reference are shown.



	0.1 g	0.23 g	0.6 g	1.3 g	3 g	Ref
one occasion	0.0%	0.0%	0.0%	1.3%	3.8%	16.3%
< 2 months (a)	0.0%	0.0%	0.0%	0.0%	0.0%	3.8%
< 2 months (b)	0.0%	0.0%	0.0%	0.0%	0.0%	13.8%
> 2 months	0.0%	0.0%	0.0%	0.0%	0.0%	10.0%

According to De Jong et al. (2010) recovery should be demonstrated on two consecutive sampling moments. Due to the 4 week interval between the last two sampling moments this would result in a very conservative effect classification. Effect class 3 was therefore subdivided in class 3a (no longer statistically significant on the last two sampling moments) and class 3b (no longer statistically significant on the last sampling moment).

At the population level three taxa showed statistically significant adverse response patterns that were considered related to the test item treatment (based on magnitude and duration in relation to dose timing). These were Poduromorpha (Collembola, 3 g a.s./ha), adult Coccinellidae (Coleoptera, 3 g a.s./ha) and adult Thysanoptera (1.3 g and 3 g a.s./ha).
For few other taxa reductions compared to the control occurred incidentally, but no consistent trend in time or relation to the dose rate was found.

Summary table effect classifications Deltamethrin EW 15 rates

Community level effects (PRC/Monte-Carlo; 5% alpha level)	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**
			Effect class		
Weed/Berlese dataset	1	1	1	1	1
Pitfall dataset	1	1	1	1	1
Suction dataset	1	1	1	1	1
Conclusion					**Community NOER**

Population level effects (Mann-Whitney U test; 5% alpha level)	**0.1 g a.s./ha**	**0.23 g a.s./ha**	**0.6 g a.s./ha**	**1.3 g a.s./ha**	**3 g a.s./ha**
			Effect class		
Poduromorpha (Arthropleona, Collembola)	1	1	1	1	2
Adult Coccinellidae (Coleoptera)	1	1	1	1	2
Adult Thysanoptera	1	1	1	2	2
Conclusion			**Population NOER**		**Population NOEAER**

NOER: No Observed Effect Rate (no statistically significant differences compared to control)

NOEAER: No Observed Ecologically Adverse Effect Rate (at least 1 taxon with effect class 2 or 3, i.e. clear response to treatment but with recovery within 2 months after application)

Effect classification:		Effect class:
no effect	No consistent treatment related statistically significant differences compared to the control	1
one occasion	Clear adverse treatment-related effect but observed only on one occasion	2
< 2 months (a)	Adverse effect no longer statistically significant on the last two sampling moments	3a
< 2 months (b)	Adverse effect no longer statistically significant on the last sampling moment	3b
> 2 months	No recovery from adverse effect within the study period (= 2 months)	8

Overall conclusions

It is concluded that Deltamethrin EW 15 applied at a rate of 3 g a.s./ha in an off-crop grassland in South-West France is the community NOER (No Observed Effect Rate).
At the population level, three taxa were adversely affected by treatment with Deltamethrin EW 15 applied at a rate of 3 g a.s./ha and one taxon by treatment with 1.3 g a.s./ha. They all recovered within one month which is considered to be the ecologically acceptable. Deltamethrin EW 15 applied at 3 g a.s./ha is therefore classified as the population NOEAER (No Observed Ecologically Adverse Effect Rate), and 0.6 g a.s./ha as the population NOER.

Report:	**KCP 10.3.2.4/03, [redacted], [redacted]; 2011**
Title:	Statement on Residues of Deltamethrin in/on Barley and Barley, Spring: Kinetic Evaluation
Document No.:	M-424226-01-1 (Rep. No.: MEF-11/879)
Guidelines:	Not applicable: Kinetic evaluation
GLP:	Not applicable

Methods:
A kinetic evaluation of the plant residue study of deltamethrin in/on barley and barley, spring in Spain, Germany, Belgium and United Kingdom ([redacted] 2011; M-408272-01-1) was performed.
The single-first-order (SFO) half-lives of deltamethrin derived in this evaluation are summarised in the table below:

Summary of DT_{50} values deltamethrin in various trials

Date	Site	Crop	DT_{50} [days]
10-2120-01	[redacted] (Spain)	barley	4.7
10-2120-02	[redacted] (Germany)	barley, spring	2.4
10-2120-03	[redacted] (Belgium)	barley, spring	2.5
10-2120-04	[redacted] (United Kingdom)	barley	2.4
Geometric mean		2.9	

Results and Conclusion:
The evaluation of the fate of deltamethrin residues on plants in different locations in Europe resulted in a DT_{50} of 2.9 days.

Supplemental information

Report:	KCP 10.2.2.4/05 [redacted] 2011; M-408272-01-1
Title:	Determination of the residues of deltamethrin and fluopicolide in/on Barley and Barley, spring after spraying of fluopicolide & fosetyl-Al WG 71 and Decis EC 025 in the field in Spain, Germany, Belgium and United Kingdom
Report No.:	10-2120
Document No.:	M-408272-01-1
Guideline(s):	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, Annex II, part A, section 6 and Annex III, part A, section 8 Residues in or on Treated Products, Food and Feed EC guidance working document 7029/VI/95 rev. 5 (1997-07-22)
Guideline deviation(s):	see page 78
GLP/GEP:	**yes**

Objective of the study

The objective of this study was to determine the magnitude of the relevant residues of fluopicolide, its metabolites (AE C657188 and AE C653711) and deltamethrin in/on barley or spring barley (green material) after one spraying application with Fluopicolide & Fosetyl-Al WG 71 a WG formulation containing 4.44 % of fluopicolide and 66.67 % of fosetyl-AL and Decis EC 025, an EC formulation containing 25 g/L of deltamethrin.

Methods

The study included four supervised residue trials conducted in northern Europe (Germany, Belgium and United Kingdom) and in southern Europe (Spain) during the 2010 season. At each trial site there was one untreated plot in addition to the treated plot(s). The treated and untreated plots were cultivated in the same manner.

The actual application data are presented in the following table. This data reflects the intended application scheme, or, if minor deviations occurred, these were within the acceptable range:

Table 26: Application summary

Trial no. Country	Formulation	Appl. mode	Reference	Application							
				No. of appl.	Interval (days)	Growth stage (BBCH code)	DBH PHI	Test item rate	Water rate (L/ha)	a.s.	Appl. rate (kg a.s./ha)
10-2120-01 Spain	Decis EC 025	SPI	GF	1	-	32	-	0.25 L/ha	600	Delta-methrin	0.006
	Fluopicolide & Fosetyl-Al WG 71							3.0 kg/ha		Fluo-picolide	0.13
										fosetyl-AL	2
10-2120-02 Germany	Decis EC 025	SPI	GF	1	-	30	-	0.25 L/ha	300	Delta-methrin	0.006
	Fluopicolide & Fosetyl-Al WG 71							3.0 kg/ha		Fluo-picolide	0.13
										fosetyl-AL	2
10-2120-03 Belgium	Decis EC 025	SPI	GF	1	-	30	-	0.25 L/ha	600	Delta-methrin	0.006
	Fluopicolide & Fosetyl-Al WG 71							3.0 kg/ha		Fluo-picolide	0.13
										fosetyl-AL	2

10-2120-04 United Kingdom	Decis EC 025	SPI	GF	1	-	30	-	0.25 L/ha	200	Delta-methrin	0.006
	Fluopicolide & Fosetyl-Al WG 71							3.0 kg/ha		Fluo-picolide	0.13
										fosetyl-AL	2

a.s.: Active substance
Appl.: Application
SPI: Spraying
DBH: Days before harvest
PHI: Pre-harvest interval
GF: Whole Area

The samples were collected in a manner designed to obtain representative samples. Each sample consisted of at least 500 g of green material for samples until 5 days after last application and at least 1 kg of green material for samples were taken more than 5 days after last application. The field samples from all trials were placed in doubled labelled bags and stored deep-frozen within 24 hours after sampling. All field samples were shipped by deep-freeze lorry under monitored conditions during shipment. The field samples were stored in a freezer at -18°C or below until preparation of the examination samples.

The analyses were conducted according to the following analytical method(s) which are described in details into the report:

Table 27: Analytical methods used

Active substance	Analytes	Method number	Limit of quantitation [mg/kg]	Measurement principle
fluopicolide	fluopicolide	01209	0.01	LC-MS/MS
	AE C657188	01209	0.01	LC-MS/MS
	AE C653711	01209	0.01	LC-MS/MS
deltamethrin	cis-deltamethrin	00855/M004*	0.05	LC-MS/MS

* the analytical method 00855/M004permits to determine cis-deltamethrin and its isomers AE F108569 and AE 0035073. For this study 10-2120, only cis-deltamethrin was quantified with this method.

Storage period of the samples:

The storage period of deep-frozen samples for fluopicolide (AG01) and its metabolites ranged between 213 and 277 days.

The storage period of deep-frozen samples for cis-deltamethrin (AG02) ranged between 252 and 311 days.

Results

The level of residues of fluopicolide, its metabolites (AE C657188 and AE C653711) and deltamethrin in the treated samples are summarised in the table below. No residues above the LOQ were found in the control samples. Results were not corrected for concurrent recoveries.

Table 28: Residue summary in/on barley or spring barley / AG01

Trial No. Country	Sample material	DALT	Residues [mg/kg]		
			fluopicolide	AE C657188	AE C653711
10-2120-01 Spain	green material	0	6.6	< 0.01	< 0.01
	green material	1	6.6	< 0.01	< 0.01
	green material	2	4.6	< 0.01	< 0.01
	green material	3	2.4	< 0.01	< 0.01
	green material	5	2.8	< 0.01	< 0.01
	green material	6	2.7	< 0.01	< 0.01
	green material	10	1.9	< 0.01	< 0.01
10-2120-02 Germany	green material	0	8.3	< 0.01	< 0.01
	green material	1	7.8	< 0.01	< 0.01
	green material	2	7.0	< 0.01	< 0.01
	green material	3	6.3	< 0.01	< 0.01
	green material	5	3.9	< 0.01	< 0.01
	green material	7	2.7	< 0.01	< 0.01
	green material	10	0.60	< 0.01	< 0.01

10-2120-03 Belgium	green material	0	6.4	< 0.01	< 0.01
	green material	1	6.8	< 0.01	< 0.01
	green material	2	5.2	< 0.01	< 0.01
	green material	3	5.7	< 0.01	< 0.01
	green material	5	3.6	< 0.01	< 0.01
	green material	7	2.6	< 0.01	< 0.01
	green material	10	0.88	< 0.01	< 0.01
10-2120-04 United Kingdom	green material	0	13	< 0.01	< 0.01
	green material	1	11	< 0.01	< 0.01
	green material	2	9.9	< 0.01	< 0.01
	green material	3	9.4	< 0.01	< 0.01
	green material	5	7.1	< 0.01	< 0.01
	green material	7	4.2	< 0.01	< 0.01
	green material	9	3.5	< 0.01	< 0.01

DALT = Days after last treatment, a.s. = Active substance

Table 29: Residue summary in/on barley or spring barley / AG02

Trial No. Country	Sample material	DALT	Residues [mg/kg] cis-deltamethrin
10-2120-01 Spain	green material	0	0.25
	green material	1	0.19
	green material	2	0.14
	green material	3	0.14
	green material	5	0.12
	green material	6	0.10
	green material	10	0.06
10-2120-02 Germany	green material	0	0.25
	green material	1	0.21
	green material	2	0.14
	green material	3	0.11
	green material	5	0.06
	green material	7	< 0.05
	green material	10	< 0.05
10-2120-03 Belgium	green material	0	0.20

	green material	1	0.17
	green material	2	0.08
	green material	3	0.10
	green material	5	0.06
	green material	7	< 0.05
	green material	10	< 0.05
10-2120-04 United Kingdom	green material	0	0.38
	green material	1	0.28
	green material	2	0.21
	green material	3	0.16
	green material	5	0.10
	green material	7	0.05
	green material	9	< 0.05

DALT = Days after last treatment a.s. = Active substance

Conclusion

The average recoveries were for all compounds and for all fortification levels within the acceptable range of 70 - 110 %, except for fluopicolide at 10 and 15 mg/kg (139% and 144%, respectively) and

for cis-deltamethrin at 1.5 mg/kg (113%). These results are considered as acceptable as they maximize the residue data found and all the results are considered as valid.

Table 30: Residues on crop after application of test item

Analyte	Crop	No of trials	Sample material	DALT	Residue [mg/kg] min - max
fluopicolide	barley	1	green material	0 - 10	1.9 – 6.6
	barley, spring	3		0 - 10	0.60 - 13
AE C653711	barley	1	green material	0 - 10	< 0.01
	barley, spring	3		0 - 10	< 0.01
AE C657188	barley	1	green material	0 - 10	< 0.01
	barley, spring	3		0 - 10	< 0.01
deltamethrin	barley	1	green material	0 - 10	0.06 - 0.25
	barley, spring	3		0 - 10	< 0.05 - 0.38

DALT = Days after last treatment

*****

Supplemental information from the literature

Report:	KCP 10.3.2.4/04; Rodrigues, R.; Goncalves, R.; Silva, C.; Torres, L.; 2004; M-462140-01-1
Title:	Toxicity of five insecticides on predatory mites (Acari: Phytoseiidae) in vineyards in two Portuguese regions
Report No.:	M-462140-01-1
Document No.:	M-462140-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

EXECUTIVE SUMMARY

To evaluate the toxicity of five insecticides on predatory mites (Acari: Phytoseiidae), two field tests were carried out during summer 2002, with a fully randomized design and five replicates per treatment, using commercial formulations at recommended field rates. Material and methods as well as results are summarized for deltamethrin (Decis® 25 g a.s./L) only.

The trials were conducted at Ponte de Lima - Minho region (trial 1) and Castelo Bran – Beira Interior region (trial 2). The test concentration (0.30 l/ha Decis®) was applied by a knapsack using a hand-lance until run-off (1000 l/ha). The control plot was treated with water.

The assessment of the mobile stages of Phytoseiid mites per leaf was performed in laboratory with a stereoscopic microscope. The leaves were detached in each replicate at five times: four days before the treatments (T0), and 4, 7, 14 and 35 days after treatments (T4, T7, Tl4 and T 35). In each assessment 25 leaves per replicate (125 leaves per treatment) were evaluated. Effects on the predatory mites were calculated with the Henderson-Tilton formula[38]. After the treatment, mean values of mobile stages of predatory mites per leaf were counted at each time of assessment in all 5 replicates and analysed statistically (ANOVA and HSD Tuckey-test).

The most abundant Phytoseiid species identified were *Phytoseius plumifer* Canest. & Fanzag (91.8%) in Minho region and *Typhlodromus phialatus* Athias-Henriot (96.7%) in Castelo Branco region

In trial 1 first significant difference between control and deltamethrin treatment were observed 4 days after of application. In the following subsequent assessments, performed 7, 14, 21 and 35 days after the treatment, the mean density of phytoseiid mites per leaf was always significantly less in the deltamethrin treatment compared to the control. The mean toxicity according to Henderson-Tilton was 95.8%.

In trial 2, predatory mites were not homogeneously distributed within the plots before the treatment. Four days after deltamethrin treatment, the density of mites decreased significantly compared to the control. However, the mean number of phytoseiids declined also in the control. In the following assessments, the mean number of mites per leaf was again significantly lower in the deltamethrin treatment compared to the control. The mean toxicity according to Henderson-Tilton was 99.5%.

[38] Henderson C.F. & Tilton E.W. 1955: Test with acaricides against brown wheat mite.- J. Econ. Ent. 48: 157-161.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Decis®
Active substance(s):	Deltamethrin (25 g/l)
Adjuvant / Surfactant:	-
Source of test item:	-
Lot/Batch number:	-
Purity:	-
Storage conditions:	-

2. Test area:

Location:	Trial 1: [redacted] Trial 2: [redacted]
Field history:	-
Cultivars:	Trial 1: Loureiro Trial 2: Rufete
Plantation density:	Trial 1: 1111 plants/ha (3 x 3 m) Trial 2: 3333 plants (2 x 1.5)
Age:	Trial 1: 9 years Trial 2: 10 years
Soil Surface:	Trial 1: Rang: herbicides, Between rows: spontaneous herbaceous species, frequent cuttings. Trial 2: Rang: herbicides Between rows: spontaneous herbaceous species, frequent cuttings.
Training system	Trial 1: Single curtain Trial 2: Double curtain
Trial area:	Trial 1: 0.35 ha Trial 2: 0.135 ha
Vines per replicate:	Trial 1: 3 Trial 2: 8
Pesticides used on fields:	-

3. Test organism(s)

Species:	Phytoseiid mites: *Phytoseius plumifer*, *Typhlodromus pyri*, *Kampimodromus aberrans*, *Euseius stipulatus*, *Typhlodromus phialatus*
Cultivar:	-
Source of test species:	-
Age of test organisms at study initiation / Crop growth stage at treatment:	-
Holding conditions prior to test:	-
Acclimatisation:	-

B. Study design and methods

1. Test procedure

Test system (study type):	Field study
Duration of study:	35 days
Treatments:	Deltamethrin and control (water)

Application rate:	0.30 l/ha Decis®
Number of replicates:	5 replicates with 125 leaves per treatment
Plot size:	-
Application / device / nozzles:	Knapsack wuth hand-lance (until run-off, 1000 l/ha)
Water volume:	-
Verification of dispersion:	-
Sampling technique:	-
Sampling frequency:	Before the treatment (T 0) and 4, 7, 14 and 28 days after treatment (T4, T7, T 14 and T 28)
Transport/storage of samples:	-

2. Environmental conditions

Soil at study site:	-
pH:	-
Organic matter (C_{org}):	-
$CaCO_3$:	-
Cation exchange capacity:	-
Soil textural fractions / extractable micronutrient concentrations [mg per kg soil]:	-
Fertilization:	-

3. Observations and measurements

Conditional (eg weather) parameters:	-
Biological parameters measured:	Density and toxicity according to Henderson-Tilton formula[38]
Measurement frequency:	Before the treatment (T 0) and 4, 7, 14 and 35 days after treatment (T4, T7, T 14 and T 35)
Statistical analyses:	Univariate variance analysis (ANOVA) and HSD Tukey-test

RESULTS

Validity criteria:

No validity criteria were mentioned.

Biological findings:

The most abundant Phytoseiid species identified were *Phytoseius plumifer* Canest. & Fanzag (91.8%) in Minho region and *Typhlodromus phialatus* Athias-Henriot (96.7%) in Castelo Branco region

Table 1: Phytoseiid species presents in terms of their abundace

Phytoseiid species	Trial 1		Trial 2	
	N	%	N	%
Phytoseius plumifer Canest. & Fanzago	1925	91.8	-	-
Typhlodromus pyri Scheuten	80	3.8	7	0.7
Kampimodromus aberrans (Oudemans)	69	3.3	25	2.6
Euseius stipulatus (Athias-Henriot)	23	1.1	-	-
Typhlodromus phialatus Athias-Henriot	-	-	922	96.7

Table 2: Toxicity classification of the insecticides, tested in Ponte de Lima (Trial 1) and Castelo Branco (Trial 2) according to the IOBC Working Group "Pesticides and Beneficial Organisms "(%=reduction in comparison to the control plot, Henderson-Tilton).

Trial area	T4 %	T7 (%)	T14 (%)	T21 (%)	T35 (%)	Tmean (%)
Trial 1	73.3	98.7	98.4	99.2	97.9	95.8
Trial 2	100	100	100	100	98.7	99.5

In trial 1, first significant difference between control and deltamethrin treatment were observed 4 days after of application. In the following subsequent assessments, performed 7, 14, 21 and 35 days after the treatment, the mean density of phytoseiid mites per leaf was always significantly less in the deltamethrin treatment compared to the control. The mean toxicity according to Henderson-Tilton was 95.8%.
In trial 2, predatory mites were not homogeneously distributed within the plots before the treatment. Four days after deltamethrin treatment, the density of mites decreased significantly compared to the control. However, the mean number of phytoseiids declined also in the control. In the following assessments, the mean number of mites per leaf was again significantly lower in the deltamethrin treatment compared to the control. The mean toxicity according to Henderson-Tilton was 99.5%.

Comment by the Notifier
The presented data confirm the known toxicity of deltamethrin to predatory mites at an application rate of 7.5 g a.s./ha conditions. Therefore, the information is classified as b) supplementary information (EFSA Journal 2011;9(2):2092).

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 for non-target soil meso- and macrofauna follows the procedure given in the Guidance Document on Terrestrial Ecotoxicology and taking into account the data requirements given in the Regulation (EC) No 1107/2009.

Predicted environmental concentrations used in risk assessment

Table 10.4- 1 Initial max PEC_{soil} values

Compound	Sugar beets (1 × 7.5 g a.s./ha)	Cauliflower (2 × 7.5 g a.s./ha)	Cereals (2 × 6.25 g a.s./ha)
	PECsoil, max [mg/kg]	PECsoil, max [mg/kg]	PECsoil, max [mg/kg]
Deltamethrin	0.0080	0.0147	0.0102
BrCA	0.0011	0.0016	0.0010
mPBacid	0.0002	0.0002	0.0001

CP 10.4.1 Earthworms

Earthworm reproduction tests (*Eisenia fetida*) were performed with the representative formulation and the metabolites Br2CA and mPBacid. The endpoints are summarized in Table 10.4.1-1.

Table 10.4.1- 1 Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
Earthworm, reproduction				
Deltamethrin EW 15	*Eisenia fetida* reproduction 56 d, mixed	NOEC	281 mg prod./kg dws 4.22 mg a.s./kg dws	[redacted] (2012) M-426439-01-1 KCA 8.4.1/03
		$NOEC_{corr.}$	140.5 mg prod./kg dws[A] **2.11 mg a.s./kg dws[A]**	
Br_2CA	*Eisenia fetida* reproduction 56 d, mixed	NOEC	10 mg/kg dws	[redacted] (2014) M-403733-01-1 KCA 8.4.1/01
		$NOEC_{corr.}$	**5 mg/kg dws[A]**	
mPBacid	*Eisenia fetida* reproduction 56 d, mixed	NOEC	10 mg/kg dws	[redacted] (2011) M-402952-01-1 KCA 8.4.1/02
		$NOEC_{corr.}$	**5 mg/kg dws[A]**	

dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected

Bold values: endpoints used for risk assessment

[A] corrected by factor of 2 due to lipophilic substance (i.e. log P_{ow} > 2)

RISK ASSESSMENT FOR EARTHWORMS

Toxicity exposure ratios for earthworms (*Eisenia fetida*) were calculated for Deltamethrin and its metabolites for the representative uses 1 x 7.5 g Deltamethrin/ha in sugar beets, 2 x 7.5 g Deltamethrin/ha in cauliflower, and 2 x 6.25 g Deltamethrin/ha in wheat (Table 10.4.1-2) considering the PECsoil values presented in Table 10.4.1.

Table 10.4.1- 2: TER calculations for earthworms

Compound	Species	Endpoint	[mg/kg]	$PEC_{soil,max}$ [mg/kg]	TER_{LT}	Trigger
Sugarbeet						
Deltamethrin EW 15	Earthworm, reproduction	NOEC	2.11	0.0080	264	5
Br_2CA	Earthworm, reproduction	NOEC	5	0.0011	4545	5
mPBacid	Earthworm, reproduction	NOEC	5	0.0002	25000	5
Cauliflower						
Deltamethrin EW 15	Earthworm, reproduction	NOEC	2.11	0.0147	144	5
Br_2CA	Earthworm, reproduction	NOEC	5	0.0016	3125	5
mPBacid	Earthworm, reproduction	NOEC	5	0.0002	25000	5
Wheat						
Deltamethrin EW 15	Earthworm, reproduction	NOEC	2.11	0.0102	207	5
Br_2CA	Earthworm, reproduction	NOEC	5	0.0010	5000	5
mPBacid	Earthworm, reproduction	NOEC	5	0.0001	50000	5

All TER values are above the critical trigger value of 5 indicating a low risk for earthworms for the intended uses of Deltamethrin EW 15. Further higher tier testing is not necessary.

CP 10.4.1.1 Earthworms - sub-lethal effects

Please refer to point KCA 8.4.1 where a chronic study with the formulation is presented.

CP 10.4.1.2 Earthworms - field studies

Considering the findings reported above no further studies are required.

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

Collembola and predatory soil mite reproduction tests (*Folsomia candida* and *Hypoaspis aculeifer*, respectively) were performed with the representative formulation and the metabolites Br_2CA and mPBacid. The endpoints are summarized in Table 10.4.2-1.

Table 10.4.2- 1 Endpoints used in risk assessment

Collembola, reproduction				
Deltamethrin EW 15	*Folsomia candida* reproduction 28 d, mixed	NOEC	178 mg prod./kg dws 2.67 mg a.s./kg dws	[redacted] (2010); M-397993-01-1 KCA 8.4.2/06
		$NOEC_{corr.}$	89 mg prod./kg dws[A] **1.34 mg a.s./kg dws[A]**	
Br_2CA	*Folsomia candida* reproduction 28 d, mixed	NOEC	≥100 mg/kg dws	[redacted] (2010) M-398826-01-1 KCA 8.4.2/03
		$NOEC_{corr.}$	**≥50 mg/kg dws[A]**	
mPBacid	*Folsomia candida* reproduction 28 d, mixed	NOEC	≥100 mg/kg dws	[redacted] (2010) M-398820-01-1 KCA 8.4.2/04
		$NOEC_{corr.}$	**≥50 mg/kg dws[A]**	
Soil mites, reproduction				
Deltamethrin EW 15	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	32 mg prod./kg dws 0.48 mg a.s./kg dws	[redacted] (2010) M-393654-01-1 KCA 8.4.2/05
		$NOEC_{corr.}$	16 mg prod./kg dws[A] **0.24 mg a.s./kg dws[A]**	
Br_2CA	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	≥100 mg/kg dws	[redacted] (2011) M-400275-01-1 KCA 8.4.2/01
		$NOEC_{corr.}$	**≥50 mg/kg dws[A]**	
mPBacid	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC	≥100 mg/kg dws	[redacted] (2011) M-400270-01-1 KCA 8.4.2/02
		$NOEC_{corr.}$	**≥50 mg/kg dws[A]**	

dws = dry weight soil; a.s. = active substance; prod. = product; corr. = corrected

Bold values: endpoints used for risk assessment

[A] corrected by factor of 2 due to lipophilic substance (i.e. log P_{ow} > 2)

RISK ASSESSMENT FOR OTHER NON-TARGET SOIL MESO- AND MACROFAUNA (OTHER THAN EARTHWORMS)

Toxicity exposure ratios for the non-target soil meso- and macrofauna (other than earthworms) *F. candida* and *H. aculeifer* were calculated for Deltamethrin and its metabolites for the representative uses 1 x 7.5 g Deltamethrin/ha in sugar beets, 2. 7.5 g Deltamethrin/ha in cauliflower, and 2 x 6.25 g Deltamethrin/ha in wheat (see Table 10.4.2-2) considering the PECsoil values presented in Table 10.4-1.

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
Sugarbeet						
Deltamethrin EW 15	*Folsomia candida*	NOEC	1.34	0.0080	168	5
	Hypoaspis aculeifer	NOEC	0.24		30	5
Br_2CA	*Folsomia candida*	NOEC	≥ 50	0.0011	≥ 45455	5
	Hypoaspis aculeifer	NOEC	≥ 50		≥ 45455	5
mPBacid	*Folsomia candida*	NOEC	≥ 50	0.0002	≥ 250000	5
	Hypoaspis aculeifer	NOEC	≥ 50		≥ 250000	5
Cauliflower						
Deltamethrin EW 15	*Folsomia candida*	NOEC	1.34	0.0147	91	5
	Hypoaspis aculeifer	NOEC	0.24		16	5
Br_2CA	*Folsomia candida*	NOEC	≥ 50	0.0016	≥ 31250	5
	Hypoaspis aculeifer	NOEC	≥ 50		≥ 31250	5
mPBacid	*Folsomia candida*	NOEC	≥ 50	0.0002	≥ 250000	5
	Hypoaspis aculeifer	NOEC	≥ 50		≥ 250000	5
Wheat						
Deltamethrin EW 15	*Folsomia candida*	NOEC	1.34	0.0102	131	5
	Hypoaspis aculeifer	NOEC	0.24		24	5
Br_2CA	*Folsomia candida*	NOEC	≥ 50	0.0010	≥ 50000	5
	Hypoaspis aculeifer	NOEC	≥ 50		≥ 50000	5
mPBacid	*Folsomia candida*	NOEC	≥ 50	0.0001	≥ 500000	5
	Hypoaspis aculeifer	NOEC	≥ 50		≥ 500000	5

All TER values are above the critical trigger value of 5 indicating a low risk for non-target soil meso- and macrofauna (other than earthworms) for all intended uses of Deltamethrin EW 15. Further higher tier testing is not necessary.

CP 10.4.2.1 Species level testing

Studies are provided under KCA 8.4.2.

CP 10.4.2.2 Higher tier testing

In view of the findings above, no higher tier testing is required.

CP 10.5 Effects on soil nitrogen transformation

Studies on nitrogen transformation in soil are available for the representative formulation Deltamethrin EW 15 (KCP 10.5/01), Deltamethrin (active substance; KCA 8.5/01) and the metabolites Br_2CA (KCA 8.5/02) and mPBacid (KCA 8.5/03). A summary of the endpoints used in the risk assessment is provided in Table 10.5-1.

Table 10.5- 1 Endpoints used in risk assessment

Test substance	Test species	Endpoint	EU agreed endpoint (Review Report 6504/VI/99-final)	Reference
Deltamethrin EW 15	Nitrogen transformation 28 d	No influence ≥0.175 kg a.s./ha **≥0.233 mg a.s./kg dws**	No	[redacted] (2010) M-396529-01-1 KCP 10.5/01
Deltamethrin		No influence ≥0.375 kg a.s./ha **≥0.5 mg a.s./kg dws**	Yes	[redacted] (1994) M-133031-01-2 KCA 8.5/01
Br_2CA		No influence ≥0.177 kg/ha **≥0.24 mg/kg dws**	No	[redacted] (2011) M-400292-01-1 KCA 8.5/02
mPBacid		No influence ≥0.177 kg/ha **≥0.24 mg/kg dws**	No	[redacted] (2011) M-400287-01-1 KCA 8.5/03

dws = dry weight soil; a.s. = active substance
Bold values: endpoints used for risk assessment

RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

In no case, deviations from the control exceeded 25% after 28 days, indicating a low risk at concentrations of up to 0.5 mg Deltamethrin/kg and up to 0.236 mg/kg of the metabolites Br_2CA or mPBacid.

Thus, it is not expected that an application of a maximum 2 × 7.5 g as/ha for the use on cauliflower will pose an unacceptable risk to non-target soil micro-organisms. This use represents the worst case use with regard to the concentration in soil (worst case PECsoil: 0.0174 mg Deltamethrin/kg, 0.0016 mg Br2CA/kg, 0.0002 mPBacid/kg; Table 10.4-1), in respect of the intended uses presented in Table 10- 1 of this dossier. Consequently potential risks of the other intended uses are covered by the assessment above.

Studies for Soil Nitrogen Transformation

Report:	**KCP 10.5/01, [redacted] (2010)**
Title:	Deltamethrin EW 15A G: Determination of effects on nitrogen transformation in soil
Document No.:	M-396529-01-1 (Rep. No.: FRM-N-150/10)
Guidelines:	OECD 216 – Nitrogen Transformation Test
GLP	GLP study

Materials and Methods:
Deltamethrin EW 15A G, analytical findings: 15.35 g/L (1.5% w/w), specification No.: 102000013165-05, batch ID: 2010-002975, master recipe ID: 0108025-001, sample description: TOX08992-00, density: 1.023 g/mL) was used in the test. A loamy sand soil was exposed for 28 d to 1.52 µL and 15.20 µL test item/kg dry weight soil. Application rates were equivalent to 1.14 L and 11.40 L test item/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.
The coefficients of variation in the control (NO_3-N) were between 1 % and 8 %. Therefore the validity criteria for the study, which requires a coefficient of variation 15 % in the control, was fulfilled.

Findings:

Effects on non-target soil microorganisms

Time Interval (days)	Application rates Deltamethrin EW 15A G				
	control	1.52 µL/kg dry weight soil		15.20 µL/kg dry weight soil	
	Nitrate-N[1)]	Nitrate-N[1)]	% difference to control	Nitrate-N[1)]	% difference to control
0-7	-1.16 ± 0.04	-1.07 ± 0.12	9 [n.s.]	-1.24 ± 0.07	9 [n.s.]
7-14	1.47 ± 0.24	1.53 ± 0.14	4 [n.s.]	1.48 ± 0.06	4 [n.s.]
14-28	1.04 ± 0.03	1.02 ± 0.09	1 [n.s.w.]	1.08 ± 0.02	1 [n.s.w.]

[1)] Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).
n.s.w = No statistically significant difference to the control (Welch-t Test for non-homogeneous variances, two-sided, α = 0.05).

Observations:
During the 28-day test, 1.52 µL Deltamethrin EW 15A G/kg dry weight soil and the 10-fold dose of the test item had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeds the trigger value of 25 %.

Conclusions:
In this test the validity criteria have been fulfilled. If used as recommended, Deltamethrin EW 15A G should not have an impact on nitrogen transformation in soils.

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 non-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas.

RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

Seedling emergence and vegetative vigour studies have been conducted with Deltamethrin EW15 (see Annex Points KCA 8.6.2).

Table 10.6- 1 Effects of Deltamethrin EW15 on non-target terrestrial plants

Number of species tested	Endpoint	Application rate	Effects	EU agreed endpoint (Review Report 6504/VI/99-final)	References
Dicotyledoneae: 8 Monocotyledoneae: 3	Seedling emergence and growth	48.5 g a.s./ha	No adverse effects	No	[redacted] 2011, M-402202-01-1, KCA 8.6.2/02
Dicotyledoneae: 8 Monocotyledoneae: 3	Vegetative vigour	48.5 g a.s./ha	No adverse effects	No	[redacted] 2011, M-402931-01-1, KCA 8.6.2/01

No phytotoxic effects > 50% were found in any of the tested plant species after the application of Deltamethrin EW 15 at the maximum application rate of 48.5 g as/ha.
The intended uses in sugarbeet, cauliflower and wheat are with maximum application rates of 7.5 g as/ha, 7.5 g as/ha and 6.25 g as/ha clearly lower than the rate tested in the seedling emergence and growth test.
It can thus be concluded that the application of Deltamethrin EW 15 under practical conditions will not pose any risk to non-target terrestrial plant species in off crop areas.

CP 10.6.1 Summary of screening data

No new studies are required.

CP 10.6.2 Testing on non-target plants

No further studies are required. Seedling emergence and vegetative vigour studies have been conducted with Deltamethrin EW15 and are summarized under KCA 8.6.2.

CP 10.6.3 Extended laboratory studies on non-target plants

Further studies were not considered necessary

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

Further studies were not considered necessary

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

No studies are required.

CP 10.8 Monitoring data

No monitoring data available.