Renewal Assessment Report

beta-cyfluthrin

Volume 3 – B.9 Ecotoxicology data

07 March 2017

Rapporteur Member State: Germany

Co-Rapporteur Member State: Hungary

Version history

When	What

Table of contents

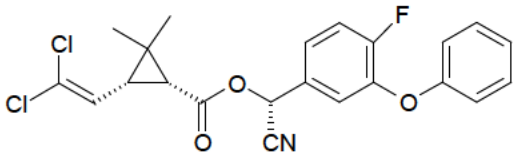
B.9 Ecotoxicology data

B.9.0 Introduction – general information about the active substance

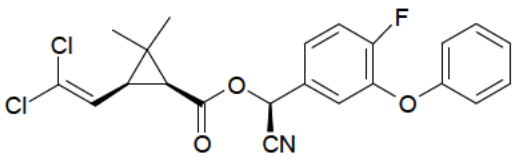
Table B 9.0-1 beta-Cyfluthrin

	Chemical name (IUPAC)	Cyano(4-fluoro-3-phenoxyphenyl)methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; or 3-(2,2-dichloro-vinyl)-2,2-dimethyl-cyclopropane-carboxylic acid cyano-(4-fluoro-3-phenoxy-phenyl)-methyl ester
	Trivial name (ISO Common Name)	beta-Cyfluthrin

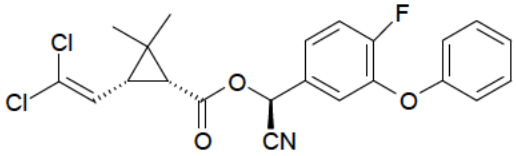
Structural formula:



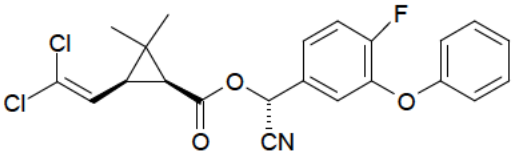
1R, 3R, alpha R



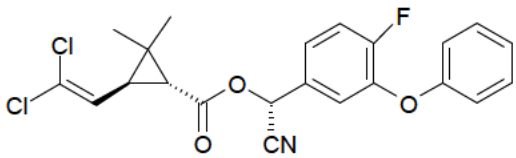
1 S, 3 S, alpha S



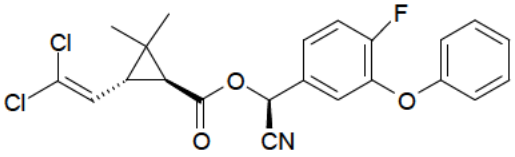
1R, 3R, alpha S



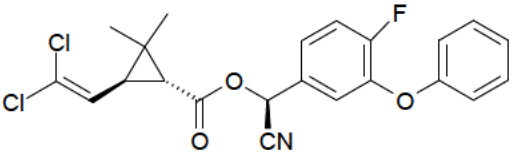
1S, 3S, alpha R



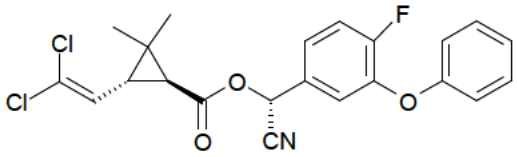
1R, 3R, alpha R



1S, 3R, alpha S



1R, 3S, alpha S



1S, 3R, alpha R

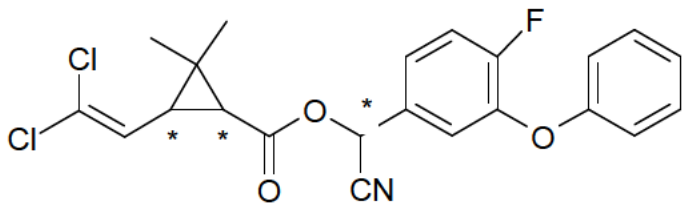
Mol. formula:	C22H18Cl2FNO3	**Mol. weight:**	434.3
Environmental compartments:	Major residue in soil, aquatic environments.		

Table B 9.0-2 Metabolites

Parent compound	Metabolite name	Compound found in	Maximal percentage of formation %
Beta-Cyfluthrin	FPB-acid	Soil	12.7 / 63.9 1
		Water	44.5 (total system)
		Sediment	24.3
	DCVA	Soil	40.5 / 75.7 1
		Water	47.6 (total system)
		Sediment	23.7
	FPB-aldehyde	Sediment	15.7

For the re-evaluation of beta-cyfluthrin studies with the active substance beta-cyfluthrin as well as studies with cyfluthrin were evaluated.

The common molecular structure of cyfluthrin and beta-cyfluthrin shows three asymmetric carbon atoms (chiral centres), which leads to four diastereoisomers each consisting of an enantiomer pair.



Thus, cyfluthrin and beta-cyfluthrin are mixtures of eight isomers.
Four of the isomers are considered active: diastereoisomer II (1R,3R,1S + 1S,3S,1R = 1:1; cis) and diastereoisomer IV (1R,3S,1S + 1S,3R,1R = 1:1; trans).

The proportion of diastereoismer pairs in cyfluthrin and beta-cyfluthrin is shown in the table below.

Diastereomer	Cyfluthrin	Beta-Cyfluthrin
I (1R-3R-R+1S-3S-S = 1:1;cis) CAS: 86560-92-1	23-27 %	< 2 %
II **(1R-3R-S + 1S-3S-R = 1:1, cis)** **CAS: 86560-93-2**	**17 -21 % (mean 19 %)**	**30-40 % (mean 35 %)**
III (1R-3R-R + 1S-3R-S = 1:1;trans) CAS: 86560-93-2	32-36 %	< 3 %
IV **(1R-3S-S + 1S-3R-R = 1:1; trans)** **CAS:** **CAS: 86560-95-4**	**21-25 % (mean 22 %)**	**57-67 % (mean 62 %)**
Sum of active diastereoismers	~ 41 %	~ 97 %
Relation of II/IV	0,86	0,56

Active diastereoismers are in written in **bold**.

Therefore, cyfluthrin is 42 % active isomers when compared to beta-cyfluthrin (41/97 = 42).
Therefore, the assumption is that the toxicity endpoints for beta-cyfluthrin should be 42 % of the cyfluthrin endpoints.
However, the relation of the two diastereoisomers II/IV is different for cyfluthrin and beta-cyfluthrin:
II/IV (cyfluthrin) = 0.86

II/IV (beta-cyfluthrin = 0.56
The relative activity/toxicity of diastereoisomers II and IV is unknown so far.
The the assumption that toxicity endpoints for beta-cyfluthrin are 42 % of the cyfluthrin endpoints can be verified either by analysing available information about the eight isomeres (four diastereoisomers) or by comparing empirical (endpoints derived from studies with beta-cyfluthrin) and predicted toxcitiy endpoints (endpoints derived from studies with cyfluthrin and transferred into beta-cyfluthrin endpoint by multiplying with 0.42) for non target organisms.

Aquatic organisms:

Acute toxicity to fish

The lowest measured acute toxicity of beta-cyfluthrin to fish (rainbow trout): **LC_{50} (4d) = 0.068 µg/L (flow –trough)**
[For details see B.9.2.1!]
Measured acute toxicity of cyfluthrin to fish (rainbow trout): **LC50 (4d) = 0.209 µg/L (flow-through)**
[This study was not submitted by the notifier. The endpoint is derived from m the US-EPA database ECOTOX.
Source: [redacted] (1994) Acute Toxicity of (Carbon 14)-Cyfluthrin to the Rainbow trout (Oncorhynchus mykiss) Under Flow-Through Conditions: Lab Project Number: BD812201: 106778. Unpublished study prepared by [redacted]. 33 p.]

Therefore, the ration between measured LC50 values (4d) of beta-cyfluthrin and cyfluthrin is **0.32** which is only slightly below the assumed factor of **0.42**.

Chronic toxicity to fish

No reliable long-term study conducted with beta-cyfluthrin is available. The previous endpoint derives from an ELS-study (flow-trough) examining the toxicity of cyfluthrin to rainbow trout:
NOEC (56 d) = 0.01 µg/L
Thus, predicted endpoint for beta-cyfluthrin is: **NOEC (56 d) =0.0042 µg/L.**
The RMS considers the application of the factor 0.42 for calculating the NOEC for beta-cyfluthrin acceptable. The observed effects in the ELS study (mortality, kyphosis, scoliosis reduced activity or erratic swimming and reduced growth) are in line with the known neurotoxic mode of action of pyrethroids. Hence, the same isomers of cyfluthrin may be responsible for acute as well as for chronic effects.

Aquatic invertebrate (chronic, acute)

Liu et al. 2005 examined the acute toxicity of every cyfluthrin isomer to *Ceriodaphina dubia* after separating by enantioselective high-performance liquid chromatography (HPLC). The study author states that the testing procedure followed the current EPA guideline (Weber CI. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Cincinnati, OH:U.S. Environmental Protection Agency; 1995.). However, the article reveals no information about the measured concentration of isomers during the course of the study. Thus, endpoints are based on nominal values.

Table of results (LC_{50} values in µg/L) copied from Liu et al. 2005:

	1*R-cis-αR*	1*S-cis-αS*	1*S-cis-αR*	1*R-cis-αS*	1*R-trans-αR*	1*S-trans-αS*	1*S-trans-αR*	1*R-trans-αS*
Cypermethrin	>7.5	>7.5	>7.5	0.775 ± 0.063	>7.5	>7.5	>7.5	0.995 ± 0.089
Cyfluthrin	>10	>10	>10	0.104 ± 0.018	>10	>10	>10	0.214 ± 0.018

Considering the results for cyfluthrin it becomes obvious that only two isomers (1R-cis-αS and 1R-trans-α-S) are responsible for toxicity to *C.dubia*.
Each of both isomers belongs to one of the two diastereoisomers II and IV, respectively.
Assuming a 1:1 distribution of enantiomers in the racemic mixures II and IV, the following proportion of the active isomers in cyfluthrin and beta-cyfluthrin is deducible:
Cyfluthrin consists of 19 % diastereomer II and 22 % diasteromer IV and consequently of 9.5 % of 1R-cis-α-cis and 11 % 1 R-trans-αS.
Beta-cyfluthrin consists of 35 % diasteromer II and 22 % diastereomer IV and consequently of 17.5 % 1R-cis-α-cis and 31 % of 1 R-trans-αS.
When assuming additive toxicity it is possible to calculate the toxicity of cyfluthrin and beta-cyfluthrin based on these portions and LC_{50} values of the two toxic isomers.

Cyfluthrin: $$\frac{1}{LC50} = \frac{0.095}{0.104} + \frac{0.11}{0.214}$$

LC_{50} = 0.700 µg/L

beta-Cyfluthrin: $$\frac{1}{LC50} = \frac{0.175}{0.104} + \frac{0.31}{0.214}$$

LC_{50} = 0.319 µg/L

$$\frac{LC50\ beta\text{-}Cyfluthrin}{LC50\ Cyfluthrin} = \mathbf{0.46}$$

Therefore, the calculated ratio is in line with the assumed 0.42.
Acutally, the LC_{50} values should be compared with real measured LC_{50} values for *Ceriodaphina dubia*. However, no data about toxicity of beta-cyfluthrin to *C.dubia* are available. Endpoints found in the ECOTOX database[1] regarding toxic effects of cyfluthrin to *C.dubia* are very inhomogeneous as derived from studies using different water qualities. Thus, they do not provide reliable endpoints. However, as the conditions for toxicity testing decribed in Liu et al. 2005 are assumed to be similar the relative toxicity of both active isomeres and consequently the LC_{50} ratio of Cylfuthrin and beta-cyfluthrin should be reliable.

Comparing acute toxicity endpoints of cyfluthrin and beta-cyfluthrin to the marine invertebrates *Americamysis bahia* the assumed ratio is not fully supported. In fact, the latter [LC_{50} (4d, beta-cyfluthrin) = 0.0022 µg/L; Machado, 1994 b, see B.9.2.3.3) is only slightly lower compared to the cyfluthrin endpoint [LC_{50} (4d, cyfluthrin) = 0.0024; Surprentant (1987), see B.9.2.3.3]. This is maybe due to deficiencies in former analytical methods. In Surpretant (1987) no limit of quantification is given. However, as two analytical methods were used (LSC and GC/ECD) results from both methods could be compared. Deviations from 27 % - 35 % were determined. However, since LC_{50} values empirically derived for cyfluthrin and beta-cyfluthrin are within the same order of magnitude, the assumed ratio of 0.42 is still regarded as acceptable.

Terrestrial vertebrates (endotherms):

The toxicity of beta-cyfluthrin and cyfluthrin for terrestrial vertebrates deviates significantly from toxicity to aquatic organisms (invertebrates, fish). Moreover, there is evidence that toxicity of beta-

[1] U.S. Environmental Protection Agency. 20XX (use current year). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. Available: http:/www.epa.gov/ecotox/

cyfluthrin and cyfluthrin to terrestrial vertebrates is in the same range. Thus, an adjustment factor is not applied.

Birds

The acute toxicity of cyfluthrin [LD_{50} > 2000 mg/kg bw; *Colinus virginianus*; [redacted] (1983), LD_{50} = 170 mg/kg bw, *Serinus canaria*, Addy-Orduna,L (2011) see B.9.1.1.1/KIIA 8.1.1/03 and B.9.1.1.1/KIIA 8.1.1/06, respectively] and the acute toxicity of beta-cyfluthrin [LD_{50} > 2000 mg/kg bw, *Colinus virginianus,* [redacted] (1994), LD_{50} =50 mg/kg bw, *Serinus canaria*, [redacted] 1985 B.9.1.1.1/KIIA8.1.1/01 and B.9.1.1.1/KIIA8.1.1/06] to birds is within the same range Studies about effects on reproduction were only submitted for cyfluthrin. The lowest ecotoxicological available end-point is consistent with the old LoEP of the first inclusion [NOEAL of 37.74 mg/kg KG/d or NOEC = 269 ppm ([redacted] et al., 1990)].

Mammals:

Please refer to Volume 3 CA B-6.

B.9.1 Effects on birds and other terrestrial vertebrates

B.9.1.1 Effects on birds

A summary of all available relevant and compliant data for beta-cyfluthrin and cyfluthrin is presented in Table B.9.1-1 to Table B.9.1-3.

Table B.9.1-1: Acute toxicity of beta-cyfluthrin and cyfluthrin to birds

species	LD_{50} (mg as/kg bw)	NOEL (mg as/kg bw)	Reference	reliability
Beta-Cyfluthrin				
Bobwhite quail *Colinus virginianus*	**> 2000**	2000	KIIA8.1.1/01 VB-027 [redacted], 1994 M-025760-01-1 R-19071	valid
Japanese quail *Coturnix coturnix japonica*	**> 2000**	< 250	KIIA8.1.1/02 VW-106 [redacted], 1985 M-053473-01-2	valid
Gallus domesticus	**> 5000**	> 5000	KIIA 8.1.1/08 [redacted], 1985, T 1019902 (FCR 4545), 13689, M-064864-01-1	valid
Canary bird *Serinus canaria*	**170**	-	KIIA 8.1.1/11 Addy-Orduna,L 2011	valid
Shiny cowbird *Molothrus bonar-iensis*	**2234**	--	KIIA 8.1.1/11 Addy-Orduna,L 2011	valid

Eared dove *Zenaida auriculata*	**2271**	-	KIIA 8.1.1/11 Addy-Orduna,L 2011	valid
Cyfluthrin				
Bobwhite quail *Colinus virginianus*	**> 2000**	2000	KIIA 8.1.1/03 426 [redacted], 1983 M-008638-01-1 R-19070	valid
Japanese quail *Coturnix coturnix japonica*	**> 5000**	500	KIIA 8.1.1/09 V-80518 [redacted], 1980 M-030215-01-3 R-19069	valid
Gallus domesticus	> 5000	< 3000	KIIA 8.1.1/04 [redacted], 1985 R3621, M-039456-01-1	not valid
Gallus domesticus	**> 3000**[1] < 5000	< 3000	KIIA 8.1.1/05 [redacted], 1985 R3622 M-039453-01-1	valid
Canary bird *Serinus canaria*	100 [2]	< 50 [2, 3]	KIIA 8.1.1/07 [redacted], 1985 VK-253 AVS 9400203 M-030284-01-1	**supporting information:** regurgitation occurred in every treatment group, bioavailablity was possibly increased due to the formulation with Cremophor EL
Canary bird *Serinus canaria*	> 125 [3]	**50**	KIIA 8.1.1/06 [redacted], 1985 VK-137 AV 94-213 M-030284-01-1	valid
Canary bird *Serinus canaria*	> 2000	< 125	KIIA8.1.1/10 EBBDL009 [redacted], 2012 M-442786-01-1 R-34708	valid, but not plausible (comprehensible);
geometric mean LD_{50} = 2939.3 mg /kg bw; **value used in the risk assessment : LD_{50} (geomean canary bird) = 92.2 mg/kg bw;** **with a safety factor of 1** Reasoning: The overall geomean LD_{50}/10 is 293.3 mg/kg bw . This value is smaller than the endpoint of the most sensitive species *Serinus canaria* (92.2 mg//kg bw).				

[1] according the study report the LD_{50} is 4500 mg/kg bw. As mortality was > 50 % at 4500 mg/kg bw, the LD_{50} is > 1000 mg/kg bw (next lower dose)
[2] as was formulated with Cremophor EL
[3] regurgitation occured
Values in **bold**: Endpoints used for risk assessment

Table B.9.1-2: Short-term toxicity of cyfluthrin to birds

Species	LD_5 (mg as/kg	NOEL	Reference	reliablity
	(mg as/kg bw)			
Beta-Cyfluthrin				
Bobwhite quail *Colinus virginianus*	> 962.5 (corresponds to 5000 ppm = highest test concentration)	192.5 (corresponds to 1000 ppm = lowest test concentration)	KIIA 8.1.2/01 428 [redacted], 1983 M-008664-01-1 R-19073	Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin and in the corresponding list of endpoints. supplemental
Mallard duck *Anas platyrhynchos*	> 567.5 (corresponds to 5000 ppm = highest test concentration)	367 (corresponds to 2000 ppm = lowest test concentration)	KIIA 8.1.2/02 421 [redacted], 1983 M-030228-01-2 R-19072	Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin and in the corresponding list of endpoints Supplemental

The studies on short-term toxicity of cyfluthrin demonstrate a low toxicity. According to the EFSA guidance (2009), a risk assessment of short-term toxicity to birds is only required under specific circumstances. It is not required in the beta-cyfluthrin assessment because of the low acute and long-term toxicity.

Table B.9.1-3: Long-term toxicity of cyfluthrin to birds

Species	Endpoint	NOEC/ NOAEC [mg as/kg feed]	NOEL/ NOAEL [mg as/kg bw/day]	Reference	Reliability
Cyfluthrin					
Bobwhite quail *Colinus virginianus*	Reproduction one generation, 23 weeks	1000	87.7	KIIA8.1.4/01 509 [redacted], 1984 M-030219-01-1 R-19075	Mentioned in the monograph for the 1. inclusion of beta-cyfluthrin and in the corresponding list of endpoints valid
Bobwhite quail *Colinus virginianus*	Reproduction one generation, 15 weeks 2	4000	321.1	KIIA8.1.4/02 654 [redacted], 1985 M-030225-01-1 R-19076	Additional information in regard to egg shell thickness
Mallard duck *Anas platyrhynchos*	Reproduction one generation, 24 weeks	250	23,8	KIIA8.1.4/03 508 [redacted], 1984 M-008671-01-1 R-19074	supplemental
Mallard duck *Anas platyrhynchos*	Reproduction one generation, 21 weeks	250	31.02	KIIA8.1.4/04 740 [redacted], 1986 M-030269-01-1 R-19077	Not valid (not plausible) (mentioned in the monograph for the 1st inclusion of beta-cyfluthrin)

Mallard duck *Anas platyrhynchos*	Reproduction one generation, 21 weeks	250	**37.74**	KIIA8.1.4/05 100359 [redacted], 1990 M-030237-01-1 R-19078	valid (mentioned in the monograph for the 1st inclusion of beta-cyfluthrin)

[1] Eggshell quality, no full one-generation
[2] Calculation described in CA 9.1.1.3

Values in bold: Endpoints used for risk assessment

B.9.1.1.1 Acute oral toxicity to birds

KIIA 8.1.1/01

Author:	[redacted]
Title:	FCR 4545 (technical grade): Acute oral toxicity to bobwhite quail
Date:	26 October 1994
Doc ID:	M-025760-01-1
Report no.:	VB-027
Guidelines:	U.S. EPA Pesticide Assessment Guideline, Subdivision E, § 71-1, dated October 1982
GLP:	Yes
Validity:	Valid

Deviations: No deviations to U.S. EPA guideline § 71-1 guideline.
The study is not conducted according to most recent OECD Guideline No. 223 (2010), but is considered valid to derive a LD_{50} value as main requirements of the OECD guideline were fulfilled and results are comprehensible.

Test material: Beta-cyfluthrin techn. (FCR 4545), purity: 98.6 %, batch no. 380466003

Test design and Methods: To test the acute toxicity of beta-cyfluthrin technical 239, 407, 692, 1176 or 2000 mg/kg bw was administered orally to groups of five male and five female bobwhite quail.

Results: The acute oral LD50 for quail orally dosed with technical grade FCR 4545 (beta-cyfluthrin) was greater than 2000 mg as/kg b.w. The no observed effect level (NOEL) was 2000 mg as/kg b.w. based on the absence of any signs of toxicity at the highest dose level.

Conclusions: LD50 > 2000 mg as/kg bw

KIIA 8.1.1/02

Author:	[redacted]
Title:	FCR 4545 Bird toxicity oral / Japanese quail (Coturnix coturnix japonica)
Date:	22 April 1985
Doc ID:	M-053473-01-2
Report no.:	VW-106
Guidelines:	U.S. EPA Pesticide Assessment Guideline, Subdivision E, § 71-1, dated October 1982
GLP:	Yes
Validity:	valid

Materials/Study design:

Test compound:	FCR 4545 (I)
Designation of the test sample:	Batch 16002/84
Technical active ingredient	
Purity:	98.5 %
Mode of administration:	gelatine capsule
Fasting period prior to administration:	3 hours
Test concentration:	250 mg/kg bw; 500 mg/kg bw; 1000 mg/kg bw; 2000 mg/kg bw
Number of animals per group:	5 female and 5 male birds
Observation period:	7 days

Results:

test concentration (mg/kg bw)	sublethal effects	mortality
250	1 x asynchronisms	0/10
500	asynchronisms, apathy	0/10
1000	asynchronisms, apathy	1/10
2000	asynchronisms, apathy	1/10

LD_{50} > 2000 mg/kg

NOEL < 250 mg/kg KG

Conclusion:
The study was conducted without control. However, no mortality is observed at the lowest test concentrations. Thus, these groups can be considered as a surrogate control group. This study is considered plausible and will be used for determing the acute toxicity endpoint.

KIIA 8.1.1/03

Author:	[redacted]
Title:	Acute oral LD_{50} of technical Cyfluthrin to bobwhite quail
Date:	15 August 1983
Doc ID:	M-008638-01-1
Report no.:	426
Guidelines:	Not reported
GLP:	no
Validity:	Valid

Materials/Study design:

Test compound:	FCR 1272 (I)
Designation of the test sample:	Batch 1030037
Technical active ingredient	
Purity:	87 %
Mode of administration:	gavage
Fasting period prior to administration:	21 hours
Test concentration:	control, 31.2 mg/kg bw; 62.5 mg/kg bw; 125 mg/kg bw; 250 mg/kg bw, 500 mg/kg bw, 1000 mg/kg bw, 2000 mg/kg bw
Number of animals per group:	5 female, 5 male
Observation period:	14 days

Deviations: In the study report no specific guideline is given. As the main criteria of OECD Guideline No. 223 are met, the study is considered reliable/valid.

Results:

test concentration (mg/kg bw)	sublethal effects	mortality
control	-	0/10
31.2	-	0/10
62.5	-	0/10
125	-	0/10
250	-	0/10
500	-	0/10
1000	-	0/10
2000	-	0/10

The acute oral LD_{50} for quail orally dosed with technical grade FCR 1272 (cyfluthrin) was greater than 2000 mg as/kg b.w. The no observed effect level (NOEL) was 2000 mg as/kg b.w. based on the absence of any signs of toxicity at the highest dose level.

Conclusions: LD_{50} > 2000 mg as/kg bw, NOEL = 2000 mg as/kg bw

KIIA 8.1.1/04

Author:	[redacted]
Title:	Acute oral toxicity (LD50) study with FCR 1272 (c.n. Cyfluthrin) vehicle: Cremophor EL 2 percent in distilled water in the hen
Date:	31 December 1985
Doc ID:	M-039456-01-1
Report no.:	R3621
Guidelines:	OECD Guidelines for Testing of Chemicals No. 401. "Acute Oral Toxicity", adopted 12.5.1981
GLP:	yes
Validity:	not valid

Material/study design:

Test compound:	FCR 1272 (I)
Vehicle:	Cremophor EL 2 % in destilled water
Designation of the test sample:	Batch 233 590 478 (Tox 1544-00)
Technical active ingredient purity:	93.5 %
Test organisms:	white Leghorn hens (laying hens), age: 12 month, 1.5 -2.1 kg bw
Mode of administration:	gavage
Fasting period prior to administration:	21 hours
Test concentration:	3000 mg/kg bw, 5000 mg/kg bw
Number of animals per group:	5 female
Observation period:	15 days

Results:

test concentration (mg/kg bw)	sublethal effects	mortality
3000	somnolence, cyanosis, emaciation, exaggerating gait	1/5
5000	somnolence, ataxia, cyanosis, emaciation	1/5

3000 mg/kg: 20 %
5000 mg/kg: 20 % (died due to malapplication)

LD_{50} > 5000 mg as/kg bw
NOEL < 3000 mg as/kg bw

Conclusion:
The study is classified as not valid since it was conducted without control. Moreover, only 5 birds were within each treatment group. Mortality in each group was 1/5 and thus, more than 10 %. Therefore, the approach to use a low-dose- treatment group as surrogate control is not applicable in this case. The study is not used for endpoint determination.

KIIA 8.1.1/05

Author:	[redacted]
Title:	Acute oral toxicity (LD50) study with FCR 1272 (c.n. Cyfluthrin) vehicle: PEG 400 in the hen
Date:	31 December 1985
Doc ID:	M-039453-01-1
Report no.:	R3622
Guidelines:	OECD Guidelines for Testing of Chemicals No. 401. "Acute Oral Toxicity", adopted 12.5.1981
GLP:	yes
Validity:	valid

Material/study design:

Test compound:	FCR 1272 (I)
Vehicle:	PEG 450
Designation of the test sample:	Batch 233 590 478 (Tox 1544-00)
Technical active ingredient purity:	93.5 %
Test organisms:	white Leghorn hens (laying hens), age: 12 month, 1.5 -2.1 kg bw
Mode of administration:	gavage
Fasting period prior to administration:	21 hours
Test concentration:	3000 mg/kg bw, 5000 mg/kg bw
Number of animals per group:	5 female
Observation period:	15 days

Results:

test concentration (mg/kg bw)	sublethal effects	mortality
3000	cyanosis, emaciation	0/5
5000	somnolence, cyanosis, emaciation	3/5

LD_{50} : > 3000 mg as/kg bw

Conclusion:
The study was conducted without control. However, no mortality is observed at the lowest test concentrations. Thus, these groups can be considered as a surrogate control group. This study is considered plausible and will be used for determing the acute toxicity endpoint.KIIA 8.1.1/06.

KIIA 8.1.1/06

Author:	[redacted]
Title:	Vogeltoxizität oral / Kanarienvogel (*Serinus canarius*) - Oral toxicity to birds / canary birds (*Serinus canarius*)
Date:	22. April 1985
Doc ID:	
Report no.:	AVS 9400203VK-252

Guidelines:	Not reported
GLP:	no
Validity:	additional information

Material/Study design:

Test compound:	FCR 1272 (I)
Vehicle:	Cremophor EL / deion. water
Designation of the test sample:	PT. 8368046/47
Technical active ingredient purity:	95.3 %
Test organisms:	canary bird (*Serinus canaries*)
Mode of administration:	gavage
Fasting period prior to administration:	1 h
Test concentration:	50 mg/kg bw, 100 mg/kg bw, 200 mg/kg bw
Number of animals per group:	5 females
Observation period:	7 days

Results:

test concentration (mg/kg bw)	sublethal effects	mortality
50	vomiting, convulsions (2)0	0/5
100	severe vomiting, apathy	2/5
200	severe vomiting, convulsions	4/5

Conclusion:
The study was conducted without control. However, no mortality is observed at the lowest test concentrations, which can be handled as a surrogate test group.
However, Cremophore is known to increase the bioavailability of xenobiotics by depressing the activity of Cytochromoxidase P450 (Rao et al. 2010)[2]. Thus, the toxicity of cyfluthrin may also be increased in this study. Indeed, canary birds react slightly more sensitively in the second study (mortality: 2/5 in the 100 mg/kg group) compared to the older study on canary birds by Hermann 1979 (mortality: 0/100 in the 100 mg/kg group). Moreover, regurgitation occurred down to the lowest test concentration of 50 mg/kg bw. This was not the case for the 50 mg/kg bw group of the first study. Due to the additive Cremophore in the second study and its effects on the bioavailability, results from this study are not used to determine the acute regulatory endpoint. However they can be considered as additional information supporting the results of Hermann 1979 as they are in the same range.

KIIA 8.1.1/07

Author:	[redacted]
Title:	Vogeltoxizität oral / Kanarienvogel (*Serinus canarius*) - Oral toxicity to birds / canary birds (*Serinus canarius*)
Date:	22.02.1979
Doc ID:	
Report no.:	AVS 94-213 VK 137
Guidelines:	Not reported
GLP:	no
Validity:	acceptable

Material/Study design:

Test compound:	FCR 1272 (I)
Vehicle:	Aceton/Plant oil

[2] Z. Rao, L. Si, Y. Guan, H. Pan, J. Qiu, G. Li, Inhibitive effect of cremophor RH40 or tween 80 - based self - microemulsiflying drug delivery system on cytochrome P450 3A enzymes in murine hepatocytes, Journal of Huazhong University of Sc ience and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban, 30 (2010) 562 – 568

Designation of the test sample:	Pt. 16002/78, Eg. 2/78
Technical active ingredient purity:	85 %
Test organisms:	canary bird (*Serinus canaries*)
Mode of administration:	gavage
Fasting period prior to administration:	1 h
Test concentration:	50 mg/kg bw, 100 mg/kg bw, 125 mg/kg bw, 250 mg/kg bw, 500 mg/kg bw, 1000 mg/kg bw
Number of animals per group:	40 femals at 1000 mg/kg bw; 20 at 500 mg/kg bw, 40 at 250 mg/kg bw, 20 at 125 mg/kg bw, 10 at 100 mg/kg bw and 10 at 50 mg/kg bw
Observation period:	7 days

Results:

test concentration (mg/kg bw)	sublethal effects	mortality (n)	mortality (%)
1000	vomiting, apathy	21/40	52,5
500	vomiting, apathy	9/20	45
250	vomiting, apathy	19/40	47,5
125	vomiting, apathy	5/20	25
100	vomiting, apathy	0/10	0
50	-	0/10	0

NOEL = 50 mg/kg bw

Conclusion:
The study was conducted without control. However, no mortality is observed at the two lowest test concentrations. At the lowest test concentration even no adverse effects were observed. Thus, these groups can be considered as a surrogate control group. This study is considered plausible and will be used for determing the acute toxicity endpoint.
According the EFSA Guidance Document 2009 part 2.1., in absence of information on the amount of regurgitated material, the lowest overall NOEL must be used for the risk assessment. Therefore, the endpoint from this study is the NOEL = 50 mg as/L.

KIIA 8.1.1/08

Author:	[redacted]
Title:	FCR 4545 techn. - Study for acute oral toxicity to the chicken (gallus domesticus)
Date:	06 August 1985
Doc ID:	M-064864-01-1
Report no.:	13689
Guidelines:	OECD Guidelines for Testing of Chemicals No. 401. "Acute Oral Toxicity", adopted 12.5.1981
GLP:	no
Validity:	valid

A study for acute oral toxicity to the chicken was conducted with the insecticidal active ingredient FCR 4545 (beta-cyfluthrin).
It conformed generally to the relevant OECD guidelines and principles.

Methods/study design:
Test organisms: white Leghorn hens (laying hens) from breeder [redacted]. The animals were about seven to ten months old, with body weights of approx. 1.4 kg to 2.1 kg
Test substance: FCR 4545 was formulated with Cremophor EL in demineralised water

(2 % v/v), and orally administered to the unfasted animals, once by stomach tube. The volume administered was 20 ml/kg body weight.

The observation period lasted 21 days.

Test compound:	FCR 4545 (beta-Cyfluthrin
Vehicle:	with Cremophor EL in demineralised water (2 % v/v)
Designation of the test sample:	Batch 16002/84)
Technical active ingredient purity:	98.5 %
Test organisms:	white Leghorn hens (laying hens), age: 10 month, 1.4 -2.1 kg bw
Mode of administration:	gavage
Fasting period prior to administration:	not fasted
Test concentration:	5000 mg/kg bw
Number of animals per group:	5
Observation period:	21 days

Results:
LD_{50} oral chicken: > 5000 mg/kg body weight
The administered dose was tolerated without signs.
There were no indications of specific organotoxic or delayed effects.
The study for acute oral toxicity therefore shows that FCR 4545 is to be seen as not toxic to the chicken.

Conclusion:
The study was conducted without control and, only 5 birds were tested at a limit dose of 5000 mg/kg. However, as no signs of toxicity occurred, this study is considered plausible and will be used for determing the acute toxicity endpoint.

KIIA 8.1.1/09

Author:	[redacted]
Title:	FCR 1272 - Acute oral toxicity to quails
Date:	06 August 1985
Doc ID:	M-030215-01-3
Report no.:	V-80518
Guidelines:	Not reported
GLP:	no
Validity:	valid

Material/Study design:

Test compound:	FCR 1272 (I)
Vehicle:	Lutrol PEG 400 (5 -25 %)
Designation of the test sample:	Batch 16001/79
Technical active ingredient purity:	no information
Test organisms:	Japanese quail (*Coturnix coturnix japonica*), bodyweigt: approx. 140 g
Mode of administration:	gavage
Fasting period prior to administration:	no information
Test concentration:	500mg/kg bw, 1000 mg/kg bw, 5000 mg/kg bw
Number of animals per group:	10 males, 10 females
Observation period:	14 days

Results:
Mortality:
males
500 mg/kg bw: 0 %
1000 mg/kg bw: 0 %
5000 mg/kg bw: 10 %

Females
500 mg/kg bw: 0 %
1000 mg/kg bw: 0 %
5000 mg/kg bw: 20 %

LD_{50} > 5000 mg/kg bw

test concentration (mg/kg bw)	sublethal effects	mortality
500	0/20	0/10
1000	6/20: apathy, ungroomed plumage, sedation	0/10
5000	7/10: apathy, ungroomed plumage, sedation	3/20 (1 male, 1 female)

LD_{50} > 5000 mg/kg bw
NOEL = 500 mg/kg bw

Conclusion:
However, no mortality is observed at the lowest test concentrations. Thus, these groups can be considered as a surrogate control group. This study is considered plausible and will be used for determing the acute toxicity endpoint.

KIIA8.1.1/10 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Toxicity of Cyfluthrin Technical During an Acute Oral LD_{50} with the Canary (*Serinus canaria*)
Date:	03 December 2012
Doc ID:	
Report no.:	EBBDL009
Edition no.:	M-442786-01-1 (R-34708)
Guidelines:	OPPTS 850.2100
GLP:	yes
Validity:	Valid, but not plausible

Deviations: none
Test material: Cyfluthrin techn., purity: 87 %, batch no. 1030037
Test design and Methods: To test the acute toxicity of cyfluthrin technical 31.2, 62.5, 125, 250, 500, 1000 or 2000 mg/kg bw was administered oraly to groups of five male and five female bobwhite quail.

MATERIALS

1. Test material:

Test item:	Cyfluthrin technical
Description:	Brown, Viscous, Crystalline
Lot/Batch #:	EBCTFK103
Purity:	94.04 %

2. Test organism:

Species:	Adult canaries (*Serinus canaria*)
Body weight of the animals:	body weights ranged from 19.2 to 23.5 g
Source:	[redacted]
Diet/Food:	Zupreme Maintenance Natural parakeet/canary/finch diet
Acclimation period:	8 weeks

3. Environmental conditions:

Temperature:	Average: 25 °C
Photoperiod:	16 hours dark, 8 hours light

STUDY DESIGN AND METHODS

1. Experimental conditions

Adult canary were orally dosed with cyfluthrin technical based on body weight at dose levels of: 0 (control), 125, 250, 500, 1000, and 2000 mg as/kg body weight. Ten birds per dose level (five males and five females) were randomised by body weight into each treatment level on experimental Day 0. Birds were capsule dosed on Day 0 and subsequently monitored for 14 days. All feed and water were provided *ad libitum*. Adult body weights were measured on experimental Day 0, Day 7, **and Day 14. Feed consumption and clinical observations occurred daily.**

2. Observations

The birds were observed twice daily (once on weekends/holidays and at study termination) during the treatment period for any mortalities and to detect any overt signs of toxicity or other clinical signs. The birds were observed three times on Day 0 following compound administration which occurred at approximately one, two, and three hours post-dosing. The study was terminated 14 days following exposure as no mortality occurred during the last three days of the 14-day observation period and no symptoms of toxicity were apparent on Day 14

3. Statistical calculations

All means and standard deviations were calculated using Excel therefore manual calculations may slightly differ. Normality and homogeneity of variance of the data were tested using the Chi-Square test ($\alpha = 0.01$) and the Levene's test ($\alpha = 0.05$), respectively. All data were normally distributed and the variances were homogenous so the data were subjected to parametric analyses. Parametric procedures involved subjecting individual body weight, body weight change, and feed consumption data to a standard one-way analysis of variance (ANOVA) followed by a means comparison using a one-tailed Dunnett's test or Bonferroni t-test (when sample sizes varied between groups; $\alpha = 0.05$ for both tests), where the means of the dose groups were compared to control means. All data in this report were analysed using parametric statistics. The statistical analyses on individual body weight, body weight change, and feed consumption data were conducted using TOXSTAT software.

RESULTS AND DISCUSSION

A. FINDINGS

The acute oral LD_{50} for cyfluthrin technical in canary was >2000 mg as/kg body weight.
Based on all parameters measured, the No Observed Adverse Effect Concentration (NOAEC) was < 125 mg as/kg body weight and the Lowest Observed Adverse Effect Concentration (LOAEC) was 125 mg as/kg body weight.

B. OBSERVATIONS

Bird Mortality

The number of bird moralities during the study were: control (0), 125 (1), 250 (1), 500 (1), 1000 (1), and 2000 (1) mg as/kg body weight. The LD_{50} was not calculated due to no treatment level having >10 % mortality.

Body Weight Change

Mean body weight changes for the periods of Day 0 to 7, Day 0 to 14, and Day 7 to 14 were subjected to hypotheses testing by sex and treatment group. Male body weight change was significantly different for Day 0 to 7 at the 1000 and 2000 mg/kg bw levels. Female body weight change was significantly different for Day 0 to 7 at the 250, 1000, and 2000 mg/kg bw levels. These effects were transient as no statistically significant body weight change occurred for any treatment level during the Day 0 to 14 or

Day 7 to 14 time-points.

Feed Consumption

Mean feed consumption change for Day 0 to 7, Day 0 to 14, and Day 7 to 14 was subjected to hypotheses testing by treatment group and sex. All feed consumption data were normally distributed and variances were homogenous therefore parametric statistical procedures were conducted with a Dunnett's test or Bonferroni t-test. Overall, male and female feed consumption data were similar between the control and all treatment groups.

Other sublethal effects

Ataxia (loss of muscular coordination) was observed in the 125 mg ai/kg body weight treatment group. Ataxia and hypo-reactivity (lethargy) was observed in the 250 and 2000 mg ai/kg body weight treatment groups. Ataxia, hypo- reactivity, and fluffed feathers was observed in the 500 mg ai/kg body weight treatment group. Ataxia, hypo-reactivity, and immobility were observed in the 1000 mg ai/kg body weight treatment group.

CONCLUSION

The acute oral LD_{50} for cyfluthrin technical in canary was >2000 mg as/kg body weight. Based on all parameters measured, the No Observed Adverse Effect Level (NOAEL) was < 125 mg as/kg body weight.

1 of 10 birds of every treatment group died shortly after administration. Moreover, the intensity of sublethal effects increased with rising application amounts of beta-cyfluthrin.

However, the exposure to beta-cyfluthrin is possibly reduced or highly variable when administered in capsules. The active substance is always administered into the birds' crop. Due to their high lipophilicity, pyrethroids might be easily absorbed via mucous membrans. A comparatively high absorption rate of pyrethroids via the whole intestinal tract in birds was shown by Edwards et al. (1986). Thus, when giving the substance directly into the birds' crop the absorption via the mucous membrane of the crop and the subsequent transport into the blood might be quicker as well as higher than when administered encapsulated. Finch birds (canary birds) have quite small crops and the amounts of liquid is less than e.g. in game birds and ducks. As sufficient liquid is necessary to resolve the gelatine capsules, these gelatine capsules possibly remain intact when passing the crop. As a consequence, the exposure to membranes in the crop might be reduced. Therefore, the effect observed in this study are less pronounced due to kind of exposure.

KIIA8.1.1/11 (new study, open access literature)

Author:	Addy-Orduna,L.; Zaccagnini, M-E.; Canavelli, S.B.; Mineau; P.
Title:	Formulated Beta-cyfluthrin Shows Wide Divergence in Toxicity among Bird Species
Date:	21.January.2011
Doc ID:	-
Source:	J. Toxicol., pp. 803451, 10 pp
Guidelines:	Draft Guideline OECD 223
GLP:	publication
Validity:	valid, reliable

Materials and Methods:

Site and General Conditions of Study

The study was carried out in the research facilities of the INTA (Instituto Nacional de Tecnología Agropecuaria) at the Paraná Agricultural Experimental Station (31°50′53″S, 60°32′19″W). The study was carried out in an aviary of 20 × 10 m, including an acclimation area with 6 groups of pens (each 3 × 2 × 3 m) and 24 individual test cages (each 0.5 × 0.5 × 0.5 m). The photoperiod and the average temperature of the testing room during the dosing were recorded (Table 9.1-6). The ventilation was con-

trolled so as to maintain the indoor conditions of temperature and humidity within outdoor environmental ranges.

Selection, Capture, and Housing of Birds
The wild birds, shiny cowbirds, and eared doves were selected based on their large numbers in surrounding fields, which assured their availability, abundance, and capture success. Shiny cowbirds were captured with mist-nets and eared doves with bait traps. Captive bred canaries were used. Healthy adult birds were weighted and grouped by sex before being acclimated to experimental conditions for at least 14 days. At least three 1.5 m-perches were placed in each pen. Shiny cowbirds were fed insectivore certified commercial food, eared doves were offered a mix of wheat and sunflower seeds, and canaries, a commercial seed mix and ground egg. Bottled water for human consumption was offered *ad libitum* to all species. Because of the absence of a constituted animal care committee at INTA or at the local university (Universidad Nacional del Litoral) which provided academic supervision of this research, guidelines of the Denver Wildlife Research Center of the US Department of Agriculture were followed for the capturing, transportation, housing, care, euthanasia, and necropsy of the birds, in addition to other procedures of the study.

Chemical and Dose
To obtain the test doses (mg beta-cyfluthrin/kg body weight), we used a commercial formulation (Bulldock of Bayer CropScience), a suspension of 12.5 g as/100 mL of unreported inert ingredients. We assumed label concentration was correctly reported and administered to birds the necessary volume of formulated product corresponding to the required dose of beta-cyfluthrin. Because wild birds are exposed to formulated products, we opted to test the formulation without an additional vehicle where possible and with distilled water as a diluent for several doses for canaries (see dilutions in the footnotes of Table B.9.1.6).
Doses were calculated according to standard equations for each stage of the approximate D-optimal design, in milligrams of as per kilogram of body weight, as shown in Table 9.1-6. The dosing volumes were calculated based on individual body weights measured within 12 hours of dosing (Table B.9.1-4). To prevent regurgitation, the higher dose volumes (>0.17 mL for canaries, >0.45 mL for shiny cowbirds, and >1.0 mL for eared doves) were split and administered in up to four aliquots separated by 15 minutes. This split administration of doses took place for all species in the limit test, one canary in the first stage of the full test and all shiny cowbirds and eared doves in all stages of the full test (Table B.9.1-4). Dose volumes never exceeded 16 mL/kg bw(body weight) in canaries, 27 mL/kgbw in shiny cowbirds, and 26 mL/kg bw in eared doves. The formulated test chemical was given by gavage. The catheter was lubricated with Vaseline to diminish possible discomfort when introduced. Individuals that regurgitated part or all of a dose and who survived the dose were substituted for others due to the fact that regurgitation modifies the dose and prevents the correct approximation of the LD_{50}. Forty-six percent of shiny cowbirds, 33 % of eared doves and 16 % of canaries regurgitated, despite being fasted before the dose.

Table B.9.1-4: Dosing volumes (mL) and number of aliquots separately administered (in brackets).

Individual	1	2	3	4	5	6	7	8	9	10
Limit test										
Canaries	0.33 [2]	0.33 [2]	0.30 [2]	0.32 [2]	0.28 [2]					
Shiny cowbirds	0.85 [3]	1.00 [3]	0.75 [3]	0.80 [3]	0.99 [3]					
Eared doves	1.97 [3]	2.13 [3]	1.86 [3]	2.26 [3]	1.79 [3]					
1st stage of the full test										
Canaries	0.12 [1]	0.13 [1]	0.16 [1]	0.24 [2]						
2nd stage of the full test										
Canaries	0.08 [1]	0.08 [1]	0.12 [1]	0.16 [1]	0.10 [1]	0.13 [1]	0.14 [1]	0.09 [1]	0.11 [1]	0.15 [1]
Shiny cowbirds	0.31 [2]	0.36 [2]	0.48 [2]	0.5 [3]	0.68 [3]	0.67 [3]	0.81 [3]	0.82 [3]	1.5 [4]	1.34 [4]
Eared doves	0.81 [2]	0.83 [2]	0.87 [2]	1.35 [2]	1.46 [2]	1.39 [2]	2.34 [3]	1.94 [3]	2.20 [3]	3.09 [3]
3rd stage of full test										
Canaries	0.12 [1]	0.10 [1]	0.10 [1]	0.10 [1]	0.09 [1]	0.13 [1]	0.13 [1]	0.13 [1]	0.14 [1]	0.16 [1]
Shiny cowbirds	0.45 [2]	0.45 [2]	0.44 [2]	0.35 [2]	0.35 [2]	1.26 [3]	1.00 [3]	1.00 [3]	1.03 [3]	1.07 [3]
Eared doves	1.44 [2]	1.24 [2]	1.42 [2]	1.02 [2]	1.24 [2]	2.88 [3]	2.77 [3]	2.66 [3]	3.09 [3]	2.09 [3]
3rd stage of the full test (2)										
Canaries	0.11 [1]	0.13 [1]	0.11 [1]	0.13 [1]	0.10 [1]	0.11 [1]	0.11 [1]	0.09 [1]		

Procedure

Acute oral toxicity tests were carried out following draft Guideline 223 of the Organisation for Economic Cooperation and Development. This procedure minimises the number of birds used and has extensive statistical validation.

First, five individuals of each species were treated with a limit dose of 2000 mg/kg of test chemical. Following any mortality at this limit dose, LD_{50} were estimated in sequential stages with the approximate D-optimal design (full test; **Figure B.9.1-1**). In canaries, the first stage of the full test was carried out to confirm and improve the initial estimate of the canary LD_{50} (250 mg/kg, based on the aforementioned literature value and the result of a limit test). An additional stage was added to obtain a greater level of precision.

Birds were randomly assigned to each test and were observed for 14 days after the dose. Mortality, clinical symptoms, change in weight between the beginning and the end of the study, regurgitations, time to death (in hours), and recovery were recorded.

Both test and control animals were examined by necropsy to determine macroscopic differences. The size, position and appearance of all organs and the full g.i. tract were examined. Also, livers and hearts were weighed and their relative weights calculated (1), in order to detect any pathology associated with any loss or increase in mass of these organs (hepatomegaly, necrosis, hypertrophy, etc.).

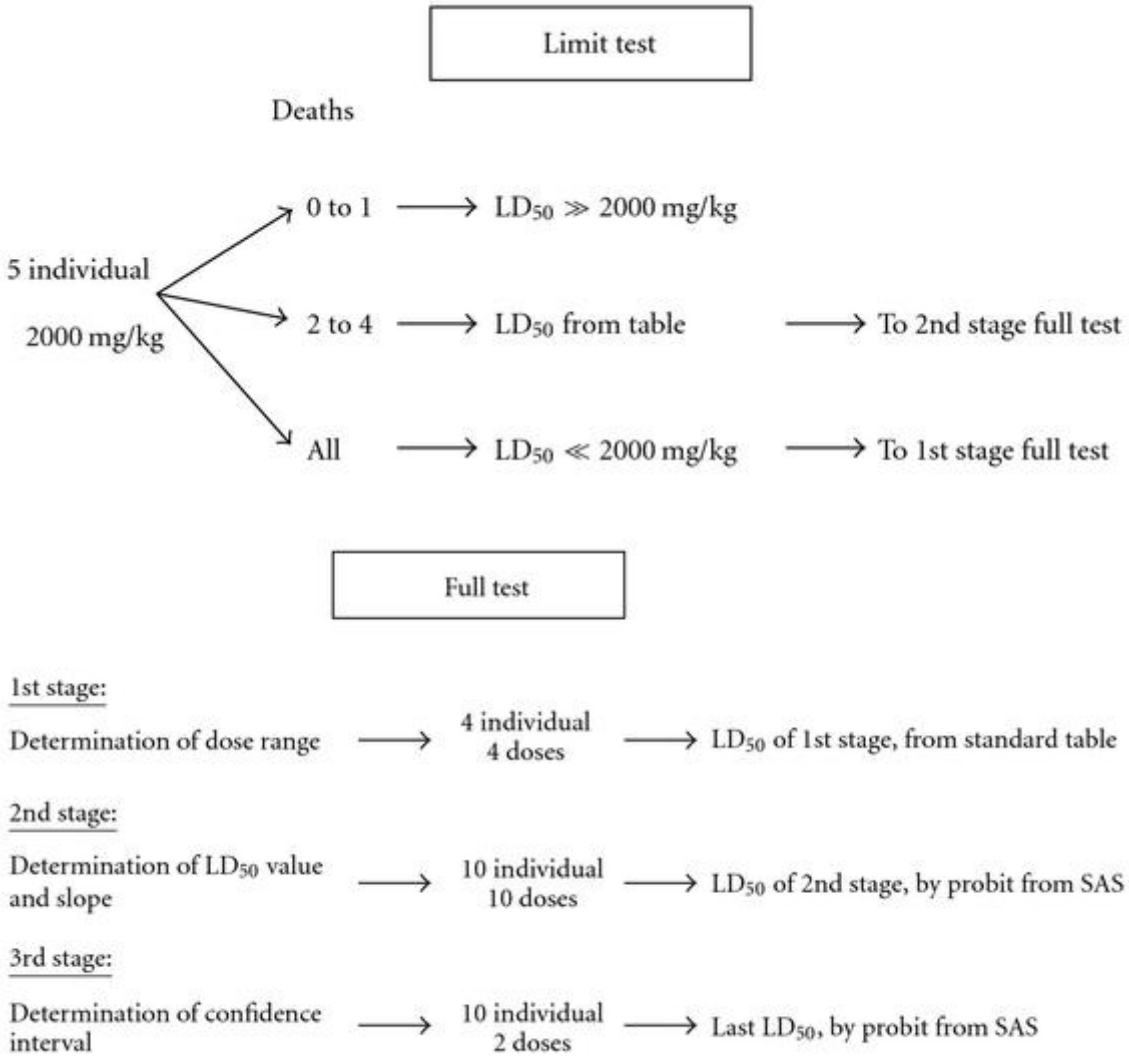


Figure B.9.1-1: Diagram of methodology used

Statistical Analysis
We fit a probit model to the combined data from all stages (STAT-SAS 6.1) to obtain the LD_{50} estimates, confidence intervals confidences and slopes of dose-response curves. Both the initial and final body weights and the relative weights of hearts and livers were compared by one-way ANOVA using SPSS v.10 for Windows.

Results

3.1. Limit Tests
Initial LD_{50} estimates obtained for the limit tests were 2247 mg/kg for both shiny cowbirds and eared doves because 40 % of individuals died in both species. By contrast, all treated canaries died, and it was, therefore, impossible to obtain an initial estimate of LD_{50} with the limit test (Table 9.1-5).

Individual	1	2	3	4	5	T (°C)	P
Canaries	X	X	X	X	X	22.6	12.7

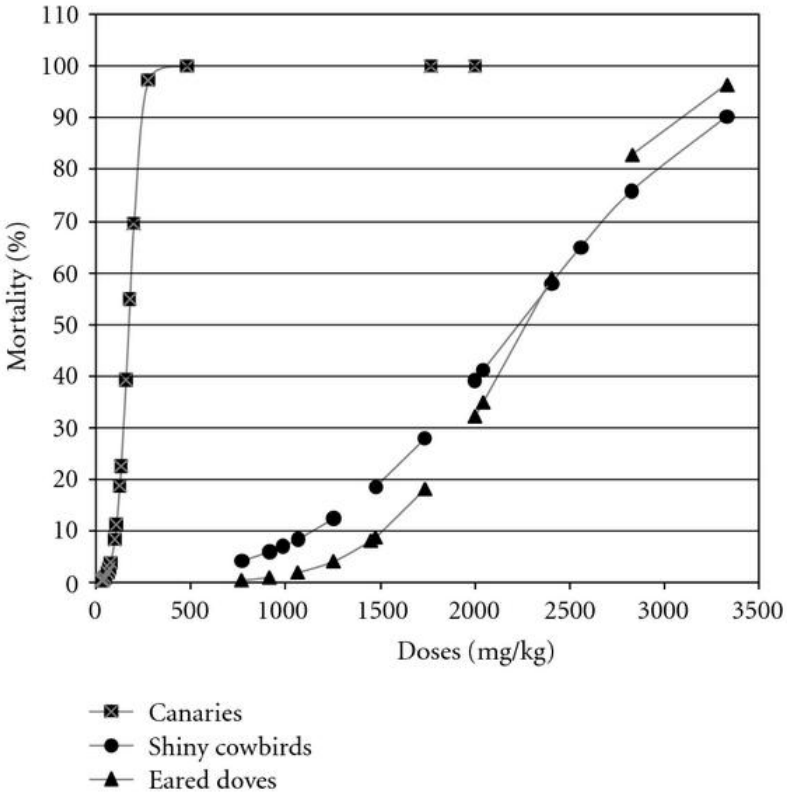
Shiny cowbirds	O†	O	O†	X	X	12.9	11.3
Eared doves	O	O†	X	X	O	19.7	11.0

X: death; O: survival; †recovered from convulsions; T: environmental average temperature during dosing; P: photoperiod, in hours of light.

Table 9.1-5: Mortality with 2000 mg/kg of test substance (limit test).

3.2. Full Test

With canaries, the LD_{50} values estimated at each sequential stage were 68 mg/kg, 110 mg/kg and 170 mg/kg, respectively. During the additional stage (similar to the third stage, performed in order to decrease the confidence intervals of the LD_{50}), two of four individuals that received the highest dose regurgitated, and, for this reason, they were not included in the results. For shiny cowbirds and eared doves, although the doses administered in the second stage were the same because of similar results in the limit test, the mortality was different (Table B.9.1-6). The LD_{50} estimates after the second stage were 1589 mg/kg and 2338.6 mg/kg for shiny cowbirds and eared doves, respectively. The final LD_{50} estimates, obtained by fitting a probit model to the combined data of all stages for each species were 170 ± 41 mg/kg for canaries, 2234 ± 544 mg/kg for shiny cowbirds, and 2271 ± 433 mg/kg for eared doves. The dose-response curves are shown in the figure below:



Clinical signs included ruffled appearance, salivation (evidenced by constant deglutition movements and head shaking), decreased activity, prostration, panting, labored breathing, body tremor, balance loss and/or convulsions. Signs appeared shortly after dosing and lasted from a few minutes to a few hours. There were doses that did not produce clinical signs and others that allowed recuperation of individuals with signs of intoxication, including convulsions (Table 9.1-5 and Table B.9.1-6). All recuperations were within the first 24 hours after the dosage. Predose and 14-day postdose weights are given in Table B.9.1-7. There were no significant differences between the body weights of the survivors before dosing and 14 days after the dose, except for canaries in the third stage of the full test where 14-day postdose weights were higher than predose weights (). Maximum time to death was 1.75 hours in canaries, 3 hours in eared doves, and 5 hours in shiny cowbirds. Only canaries showed a tendency toward a shorter time to death with increasing dose.

Table B.9.1-6: Mortality in full test

Individual	1	2	3	4	5	6	7	8	9	10	T(°C)	P
1st stage												
Dose (mg/kg)	35.4[a]	130.2[b]	479.8[g]	1767.8								
Canaries	O	X	X	X							24.2	12.7
2nd stage												
Dose (mg/kg)	23.2[b]	29.5[b]	37.4[b]	47.5[b]	60.2[c]	76.4[c]	79.0[e]	123.0[e]	156.1[e]	198.0[e]		
Canaries	O*	O*	O*	O*	O	O	O[†]	X	X	X	25.8	12.8
Dose (mg/kg)	769.6	976.5	1065.7	1254.1	1475.7	1736.6	2043.5	2404.8	2829.8	3330.0		
Shiny cowbirds	O*	O*	O	X	O	O	X	X	X	X	15.1	11.0
Eared doves	O*	O*	O	O	O	X	O	X	O	X	19.9	11.3
3rd stage												
Dose (mg/kg)	68.3[c]	68.3[c]	68.3[c]	68.3[c]	68.3[c]	177.2[e]	177.2[e]	177.2[e]	177.2[e]			
Canaries	O	O	O	O	O	O	O	X	O	O	22.6	12.8
Dose (mg/kg)	985.5	985.5	985.5	985.5	985.5	2558.9	2558.9	2558.9	2558.9	2558.9		
Shiny cowbirds	O	O	O	O	O[†]	O	X	O[†]	X	O	27.4	11.2
Dose (mg/kg)	1451.0	1451.0	1451.0	1451.0	1451.0	3330.0	3330.0	3330.0	3330.0	3330.0		
Eared doves	O	O	O	O	O	X	X	X	X	X	21.5	12.0
3rd stage (2)												
Dose (mg/kg)	105.5[d]	105.5[d]	105.5[d]	273.9[f]	273.9[f]	273.9[f]	273.9[f]	273.9[f]				
Canaries	O	O	O	O	_	X	_	X			19.9	12.9

Dilutions:[a]0.04 ,[b]0.05 ,[c]0.1, [d]0.15,[e]0.2,[f]0.4;[g]0.5 *without clinical signs of intoxication; X: death; O: survival; [†]recovered from convulsions;T : environmental average temperature during dosing;P : photoperiod, in hours of light

Table B.9.1-7: Body weights (±0.05 g for canaries, ±0.1 g for shiny cowbirds and eared doves) - weights are given as predose weight—14-day postdose weight

Test	Individual	Canaries	Shiny cowbirds	Eared doves
Limit test	1		53.0–47.4	118.0–128.0
	2		62.2–54.2	127.5 –126.0
	3		46.8–39.6	
	4			
	5			107.2–112.0
1st stage of the full test	1	17.40–21.00		
	2			
	3			
	4			
2nd stage of the full test	1	22.75–21.95	50.0–57.0	131.6–130.0
	2	17.85–19.85	46.0–56.5	105.9–115.0
	3	20.30–23.55	56.1–61.0	101.5–100.0
	4	21.40–24.60		134.5–122.0
	5	20.15–21.00	57.6–57.8	124.0–122.0
	6	20.55–21.10	48.0–52.8	
	7	18.65–21.10		143.0–133.3
	8			
	9			97.0–98.0
	10			
3rd stage of full test	1	21.60–22.35	57.2–52.5	124.0–128.0
	2	17.90–23.35	57.0–52.0	107.0–110.0
	3	18.60–21.00	56.2–54.0	122.0-123.0
	4	18.60–22.10	45.0–44.0	88.0–116.0
	5	17.10–18.85	44.7–45.0	107.0-106.0
	6	17.85–18.60	61.5–55.0	
	7	18.25–20.80		
	8		49.0–46.0	
	9	20.35–27.35		
	10	22.10–23.10	52.4–50.0	
3rd stage of the full test (2)	1	19.95–23.35		
	2	22.95–24.60		
	3	20.40–23.00		
	4	23.40–23.60		
	6			
	8			

All birds that died presented stiffness of fore and back limbs. We observed a white thick liquid in different sections of the g.i. tract, attributable to the insecticide formulation. Macroscopic differences among organs of treated and control individuals were not detected. Relative weights of heart and liver (and) did not vary either (in all cases).

Conclusion:

LD_{50} for canaries = 170 mg as/kg bw
LD_{50} for shiny cowbirds = 2234 mg as /kg bw
LD_{50} for eared doves = 2271 mg as/kg bw

Based on information provided by the published article the study is considered valid and plausible. The possible influence of other ingredients in the formulation was discussed in the publication. It is argued that the relative lack of toxicity in the other two species (with LD_{50} values similar to those obtained in quail with the active ingredient alone) suggests that differences in the sensitivity are due to the pyrethroid an not to the inerts included in the formulated material.

Discussion on discrepancies between the outcome of the different acute toxicity studies on canary birds:

Comparing the determined LD50 values from studies on canary birds, strong discrepancies become obvious. [redacted] 1979 and [redacted] 1985 concluded LD_{50} values of > 125 mg/kg and 100 mg/kg. The latter study was conducted with Cremophore. It is known to increase the bioavailability of xenobiotics by depressing the activity of Cytochromoxidase P450 (Rao et al. 2010)[3]. Thus, the toxicity of beta-cyfluthrin may also be increased in this study. Indeed, canary birds react slightly more sensitively in the second study (mortality: 2/5 in the 100 mg/kg group) compared to the first study (mortality: 0/100 in the 100 mg/kg group). Moreover, in the second study, regurgitation occurred down to the lowest test concentration of 50 mg/kg bw. This was not the case for the 50 mg/kg bw group of the first study. Due to the additive Cremophore in the second study and its effects on the bioavailability, results from this study are not used to determine the acute regulatory endpoint. However they can be considered as additional information supporting the results of [redacted] 1979 as they are in the same range. According the EFSA Guidance Document 2009 part 2.1., in absence of information on the amount of regurgitated material, the lowest overall NOEL must be used for the risk assessment. Thus, the endpoint from the study Hermann 1979 is NOEL = 50 mg as/L.

The new study on canary birds conducted with cyfluthrin [redacted] 2012) contradicts the results from [redacted] 1979 and the supporting study ([redacted], 1985).
The concluded LD_{50} is > 2000 mg/kg bw, although the NOEL concerning sublethal effects and mortality is < 125 mg/kg bw. 1 of 10 birds of every treatment group died shortly after administration. However, the intensity sublethal effects increased with rising applied amounts of beta-cyfluthrin.

The big difference (factor 40) between endpoints of both studies on the same species can't be explained by intra species deviation. Therefore, either the high or the low endpoint is questionable.

The high sensitivity of canary birds as an outcome of [redacted] (1979) and the supporting study [redacted] (1985) was also observed in the published study Addy-Orduna (2011). This study was conducted with the beta-cyfluthrin formulation Bulldock 125 SC on three bird species (canary birds *Serinus canaries,* shiny cowbirds *Molothrus bonareinsis* and eared dove *Zenaida auriculata*) according to the draft OECD Guideline 223. Based on information provided by the published article the study is considered valid and plausible. A LD_{50} of 170 mg/kg bw was determined. The possible influence of other ingredients in the formulation was discussed in the publication. It is argued that the relative lack of toxicity in the other two species (with LD_{50} values similar to those obtained in quail with the active ingredient alone) suggests that differences in the sensitivity are due to the pyrethroid an not to the inerts included in the formulated material.

Discrepancies between results from the study by [redacted] (1979) supported by [redacted] (1985) and confirmed by Addy-Orduna (2011) and [redacted] (2012) may be caused in the different way of

[3] Z. Rao, L. Si, Y. Guan, H. Pan, J. Qiu, G. Li, Inhibitive effect of cremophor RH40 or tween 80 - based self - microemulsiflying drug delivery system on cytochrome P450 3A enzymes in murine hepatocytes, Journal of Huazhong University of Sc ience and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban, 30 (2010) 562 – 568

administration. Whereas the test substance in [redacted] (1979), [redacted] (1985) and Addy-Orduna (2011) was applied by gavage, it was given in a gelatine capsule in the study by [redacted] (2012). Both types of application are acceptable according to OECD guideline 223. However, the exposure to beta-cyfluthrin is possibly reduced or highly variable when administered in capsules. The active substance capsuled or not is given into the birds' crop. Due to their high lipophilicity, pyrethroids might be easily absorbed via mucous membrans. A comparatively high absorption rate of pyrethroids via the whole intestinal tract in birds was shown by [redacted] (1986). Thus, when giving the substance directly into the birds' crop the absorption via the mucous membrane of the crop and the subsequent transport into the blood may be quicker as well as higher than when applied encapsulated. Finch birds (canary birds) have quite small crops and the amounts of liquid that is necessary to resolve the gelatine capsules are less than e.g. in game birds and ducks. Thus, gelatine capsules remain more or less intact when passing the crop; the exposure to membranes in the crop is reduced.
Thus, especially for lipophilic substances and their administration to small finch birds, applications via capsules might be not appropriate to gain reliable toxicity results.

Conclusion: Results about morality by [redacted] (2012) are not plausible in the context of all available toxicity studies on canary birds conducted with cyfluthrin or (beta-) cyfluthrin formulations. The big deviations (factor 12- 40) cannot be explained by intra-species variations.
[redacted] (2013) applied the substance within a gelatine capsule. In the other three studies, the substance was directly given into the crop by gavage. Due to the high lipophilicity of cyfluthrin and beta-cyfluthrin it might be assumed that high proportions of the substances are already absorbed in the crop if applied directly. Due to comparatively low amounts of liquid in the crop of finch birds, the gelatine capsule remains probably more or less intact. Thus, the exposure to crop membranes is reduced. Consequently, results of [redacted] (2012) do not deliver reliable endpoints concerning mortality and can't be used to deduce the regulatory endpoint for acute toxicity to birds.
The geometric mean between the NOEL of [redacted] 1979 and Addy-Orduna (2011) is used for determing the LD_{50} for canary birds. Geomean LD50 =92 mg/kg bw.

B.9.1.1.2 Short-term dietary toxicity to birds

Under Regulation 1107/2009 as well as in the current guidance document on Risk Assessment for Birds and Mammals (EFSA 2009) short-term toxicity data for birds are not required. However, old studies with cyfluthrin conducted with bobwhite quail ([redacted], 1983) and with mallard duck ([redacted], 1983) are available and were used for Annex I inclusion of beta-cyfluthrin. The studies are regarded to be valid and appropriate for the Renewal of Approval of beta-cyfluthrin. The relevant endpoints are summarised in Table B.8.1-2.

KIIA 8.1.2/01

Author:	[redacted]
Title:	Acute dietary LC_{50} of Cyfluthrin technical to bobwhite quail
Date:	18 August 1983
Doc ID:	
Report no.:	428
Edition no.:	M-008664-01-1 (R-19073)
Guidelines:	OECD Guideline No. 205 and US-EPA FIFRA § 71-2 guideline
GLP:	no
Validity:	Not valid / supplemental

Deviations: The study report is only a summary, no raw data are available (e.g. data about as concentration in feed) plausible in regard to effects
Material: Cyfluthrin techn., purity: 87 %, batch no. 1030037
Results and Discussion: The dietary LC_{50} and the lowest lethal concentration of cyfluthrin technical to bobwhite quail were greater than 5000 mg as/kg diet.

The NOEC was 1000 mg as/kg diet.
Conclusion: supplemental data

KIIA 8.1.2/02

Author:	[redacted]
Title:	Acute dietary LC50 of Cyfluthrin technical to mallard ducks
Date:	11 August 1983
Doc ID:	M-030228-01-2
Report no.:	421
Guidelines:	OECD Guideline No. 205 and US-EPA FIFRA § 71-2 guideline
GLP:	no
Validity:	Not valid / supplemental

Deviations: The study report is only a summary, no raw data are available (e.g. data about as concentration in feed) plausible in regard to effects
Materials: Cyfluthrin techn., purity: 87 %, batch no. 1030037
Study design and methods: Test diets containing 0, 2000 or 5000 mg as/kg diet cyfluthrin technical as were fed to the adults for 5 days.
Results and Discussion: The dietary LC_{50} of cyfluthrin technical to mallard duck was greater than 5000 mg as/kg diet and the lowest lethal concentration was 5000 mg as/kg diet.
The NOEC was 2000 mg as/kg diet.
Conclusion: supplemental data

Conversion of avian dietary study results from feed concentration to daily dose
Results of avian short-term dietary studies have been converted to daily dose (mg/kg bw) as recommended in 'Guidance of EFSA, Risk Assessment for Birds and Mammals', European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2009: 7(12):1438. A summary of the daily dose for each treatment level for the two short-term dietary exposure dietary studies with cyfluthrin is depicted below.
The daily dose for birds in each treatment group of each test, expressed as test substance (TS) intake, was calculated by treatment group using the following formula:
Test substance intake (mg TS/g bw/day) = $(Consumption_{mean} \times Conc_{Feed}) / BW_{mean}$
$Consumption_{mean}$= Group Mean Feed Consumption (g/bird/day)
$Conc_{Feed}$ = Concentration (mg TS/kg feed);
BW_{mean}= Group Mean Body Weight for Start of Treatment and Exposure Termination (g)
The values used in the calculations and the daily dose values are presented in the tables below.

Dietary Dose Level (mg as/ kg feed)	Group Mean Feed Consumption (g/bird/day)	Group Mean Body Weight (g)	Daily Dose (mg as/kg bw/day)
Bobwhite quail dietary study (days 0 and 5)			
Control	8.6	40 [1]	0
1000	7.7	40 [1]	192.5
5000	7.7	40 [1]	962.5
Mallard duck dietary study (days 0 and 5)			
Control	68.5	241 [2]	0
2000	49.1	263 [2]	373.4
5000	31.1	274 [2]	567.5

1 estimated mean weight
2 based on information described in the appendix of the study (derived from the raw data)

B.9.1.1.3 Sub-chronic toxicity and reproduction to birds

Annex I inclusion of beta-cyfluthrin is based on studies with cyfluthrin. The possible effect of long-term (15 to 24 weeks) uptake on the reproduction of birds was assessed. Studies were performed with the mallard duck ([redacted], 1984; [redacted], 1986; [redacted] 1990) and with the bobwhite quail

([redacted], 1984; [redacted], 1985). The studies are regarded to be valid and appropriate for the Renewal of Approval of beta-cyfluthrin. A short summary of the studies is given below.

Long-term/reproduction studies with bobwhite quail

KIIA8.1.4/01

Author:	[redacted]
Title:	Effect of Cyfluthrin (Baythroid technical) on bobwhite quail reproduction
Date:	9 August 1984
Doc ID:	M-030219-01-1
Report no.:	509
Guidelines:	US-EPA FIFRA § 71-3 guideline
GLP:	yes
Validity:	valid

Deviations: None, study is valid according to OECD Guideline No. 206
Material: Cyfluthrin technical, purity: 90.5 %, batch no. 3-03-0143 (reference #83-R-132-91)
Test design and Methods: One-generation study. Test diets containing 0, 250, 1000 or 4000 mg as/kg diet Cyfluthrin technical as were fed to the adults for 23 weeks.

Results and Discussion: No clinical signs of intoxication were observed. One control and two birds in the top dose of 4000 mg as/kg diet died during the study. However, it was reported that this was not related to the compound. In the 4000 mg as/kg diet group, threshold effects were observed on adult male body weight (no effects on females), eggs laid and hatching percentages. Accordingly, the no observed effect concentration (NOEC) was 1000 mg as/kg diet. Significant effects on egg shell thickness were seen in all treatment groups. However, the reduction was more over time than related to concentration and was also seen in the control group. Therefore, the effects were not attributed to the treatment. It was claimed that the values were well within the range published in OECD Guideline 8, Avian Reproduction Test. 1982.
Possible effects on eggshell were investigated further in the following study.
Conclusion: NOEC = 1000 mg as/kg diet; NOEL = 87.7 mg/kg bw/d

KIIA8.1.4/02

Author:	[redacted]
Title:	Effects of Cyfluthrin (Baythroid technical) on bobwhite quail eggshells
Date:	7 August 1985
Doc ID:	
Report no.:	654
Edition no.:	M-030225-01-1 (R-19076)
Guidelines:	US-EPA FIFRA § 71-3 guideline
GLP:	yes
Validity:	Additional information

Deviations: The study design was limited to determine effects on eggshell thickness. The study is supplemental to the study by [redacted] (1984, report no. 509).
Material: Cyfluthrin techn., purity: 90.5 %, batch no. 3-03-0143 (reference #83-R-132-91)
Test design and Methods: One-generation study. Test diets containing 0, 10, 45, 200, 900 or 4000 mg as/kg diet cyfluthrin technical were fed to the adults for 15 weeks. The study design was limited to determine effects on eggshell thickness. Effects on the no. of fertile eggs, viable 3-week embryos hatchling success, no. of hatchlings, no. 14 day old survivors, and hatch as well as survivors weight

were not examined.
Results and Discussion: No clinical sign of intoxication were observed. Two birds at 10 mg as/kg died during the study. However, it was reported that this was not attributed to the treatment. Decreases in body weight gains at 4000 mg as/kg diet was observed in bobwhite quail at the end of the study. No effects on egg production, eggshell thickness or eggshell strength were seen. Therefore, the overall no-effect concentration was 900 mg as/kg diet and the no effect concentration (eggshell-thickness) was 4000 mg as/kg diet.
Conclusion: supplemental information

Long-term/reproduction studies with mallard duck

KIIA8.1.4/03

Author:	[redacted]
Title:	Effects of Cyfluthrin (technical Baythroid) on mallard duck reproduction
Date:	9 August 1984
Doc ID:	M-008671-01-1
Report no.:	508
Guidelines:	US-EPA FIFRA § 71-3 guideline
GLP:	yes
Validity:	Not valid/ supplemental information

Deviations: Not valid. The validity criterion "a minimum of 14 hatchlings / hen" in the control group was not met. Moreover several other determined control parameters were beneath thresholds according OECD Guideline No. 206, e.g. no. of viable embryos/eggs set was at 61 % instead of 85 -98 %, the no. hatchlings/eggs set was only at 31.9 % instead of 50-90 %. Furthermore, the reproduction success of the control group showed high variability. Nevertheless, effects on reproduction of the 1000 ppm treatment group were still statistically different from the control. Therefore, the results of the study are considered as supplemental information to the study by Beavers (1990).
Material: Cyfluthrin techn. (Baythroid), purity: 90.5 %, batch no. 3-03-0143 (reference #83-R-132-91)
Study design and methods: One-generation study. Test diets containing 0, 250, 1000 or 4000 mg as/kg diet cyfluthrin technical were fed to the adults for 24 weeks
Results: No birds died or exhibited signs of intoxication when exposed to up to 4000 mg cyfluthrin/kg diet for 24 weeks. Adult mallard ducks showed decreased weight gains over the first four weeks. Reduced egg production and embryo viability and hatchability occurred at dietary concentrations of 1000 and 4000 mg as/kg diet. Accordingly, the NOEC was 250 mg as/kg diet.
Conclusion: supplemental information to the study by Beavers (1990)

KIIA8.1.4/04

Author:	[redacted]
Title:	Baythroid Technical: A one-generation reproduction study with the mallard (Anas platyrhynchos)
Date:	10 April 1986
Doc ID:	M-030269-01-1
Report no.:	740
Guidelines:	US-EPA FIFRA § 71-3 guideline and OECD Guideline No. 206
GLP:	yes
Validity:	Not valid

Deviations: Not valid.

The validity criterion “a minimum of 14 hatchlings / hen” in the control group (only 7/hen) was not met. Furthermore the no. of eggs laid/hen in the control group was too small only 26 instead of 28-38. Besides, the reproduction success (eggs laid/hen) of the control group showed high variability (standard deviation of 61 %).

Material: Cyfluthrin techn. (Baythroid), purity: 94 %, batch no. 5030162
Test design and Methods: One-generation study. Test diets containing 0, 10, 50 or 250 mg as/kg diet cyfluthrin technical were fed to the adults for 21 weeks.
Results: Dietary concentrations of cyfluthrin (Baythroid technical) of up to 250 mg as/kg diet did not result in treatment related mortality or overt signs of toxicity among mallards during the 21-week exposure period. There were no apparent treatment related effects upon body weight or feed consumption among adults or body weight of hatchlings at any of the concentrations tested. There was no treatment related effect upon any reproduction parameters at concentrations of up to 250 mg as/kg diet of cyfluthrin. The no-observed-effect concentration for cyfluthrin in this study was 250 mg as/kg diet, the highest concentration tested.
Conclusion: not valid

KIIA8.1.4/05

Author:	[redacted]
Title:	Baythroid Technical: A one-generation reproduction study with the mallard (Anas platyrhynchos)
Date:	20 September 1990
Doc ID:	
Report no.:	100359
Edition no.:	M-030237-01-1 (R-19078)
Guidelines:	US-EPA FIFRA § 71-3 guideline and OECD Guideline No. 206
GLP:	yes
Validity:	valid

Deviations: None, the study is valid according to OECD Guideline No. 206
Material: Cyfluthrin techn. (Baythroid), purity: 94 %, batch no. 5030162
Study design and Methods: One-generation study. Test diets containing 0, 10, 50 or 250 mg as/kg diet cyfluthrin technical were fed to the adults for 21 weeks.
Results and Discussion: Dietary concentrations of cyfluthrin (Baythroid technical) of 10, 50 or 250 mg as/kg diet did not result in treatment-related mortality or overt signs of toxicity among adult mallards during the exposure period of approximately 21 weeks. There were no apparent treatment-related effects upon body weight, feed consumption or reproductive parameters at dietary concentrations of up to 250 mg as/kg diet of cyfluthrin. Accordingly, the NOEC in this study was 250 mg as/kg diet (corresponding to a mean measured concentration in feed 269 mg as/kg feed), the highest concentration tested.

Conclusion: NOEC = 269 mg as/kg feed

Overall conclusion on reproduction studies

In bobwhite quails, the lowest obtained NOEC of cyfluthrin was 1000 mg as/kg feed ([redacted] 1984, report no. 509). The second study ([redacted], 1985, report no. 654) was conducted to investigate possible effects on eggshells seen in the first study. This study confirms that there were no effects on eggshell strength and thickness. The overall NOEC for the bobwhite quail was 1000 mg as/kg feed.

In mallard ducks, the NOEC of cyfluthrin was 269 mg as/kg feed (mean measured corresponding to 250 ppm nominal) in the only valid study [Beavers (1990)]. This value corresponds with the maximum treatment concentration. However, despite of the low reproduction success and high variability in con-

trol in the study by [redacted] (1984) statistically significant effects were observed at concentrations of 1000 mg as/kg and 4000 mg as/ kg feed. That confirms that the NOEC is equal to and not greater than 269 mg as/kg feed (mm) [250 mg as/kg feed (nom)].

In conclusion, the endpoint for the long-term/reproductive risk assessment for birds is derived from the only valid study by [redacted] *et al*. (1990, report 100359) with Mallard ducks. This endpoint is confirmed with information from the study by [redacted] (1983).

Furthermore, this endpoint is also the current EU agreed endpoint.

Conversion of avian reproduction study results from feed concentration to daily dose

Table B.8.1-6: Daily dose conversion from cyfluthrin avian reproduction studies

Nominal dose (mg as/ kg feed)	**Group Mean Feed Consumption (g/bird/day)**	**Group Mean Body Weight (g)**	**Daily Dose (mg as/kg bw/day)**
Bobwhite quail dietary study (report no. 509)			
Control	19.2	206	0
250	18.6	205	22.7
1000	17.8	203	87.7
4000	18.1	202	358.4
Mallard duck dietary study (report no. 100359)			
Control	148.9	1136	0
10	156.1	1160	1.3
50	142.4	1133	6.3
250*	161.5	1151	35.1

* this corresponds to 269 mg as/kg feed (mean measured)

Taking data from table Table 8.1-6 into account a **NOEL of 37.74 mg as /kg bw/day** is calculated from the **NOEC of 269 mg/kg feed** .

B.9.1.2 Effects on terrestrial vertebrates other than birds

B.9.1.2.1 Acute oral toxicity to mammals

Acute oral toxicity studies with rodents that have already been evaluated for the Annex I inclusion of beta-cyfluthrin as well as a new study are summarised in the table below. For details please refer to document Vol 3_CA_B 5.2.1

Animal species	Sex	Formulation agent	LD50 (mg as/kg bw)	References
rat	male*	PEG 400 #	380	Heimann, 1987b
	female*	PEG 400	651	Heimann, 1987b
	male	PEG 400	655	Heimann, 1987b
	female	PEG 400	1369	Heimann, 1987b
	male*	xylene	211	Heimann, 1987c
	female*	xylene	336	Heimann, 1987c
	male	xylene	307	Heimann, 1987c
	female	xylene	343	Heimann, 1987c
	male*	acetone/oil 1:10	84	Heimann, 1987d
	female*	acetone/oil 1:10	77	Heimann, 1987d
	male	acetone/oil 1:10	141	Heimann, 1987d
	female	acetone/oil 1:10	108	Heimann, 1987d
	female*	acetone/oil 1:10	200	Schüngel, 2005a
	male*	water/Cremophor EL	11	Heimann, 1986a
mouse	male*	PEG 400	91	Heimann, 1987e
	female*	PEG 400	165	Heimann, 1987e

* = fasted animals
# = PEG 400: polyethylene glycol 400 (Lutrol)

The acute oral toxicity of beta-cyfluthrin depends on the vehicle used. Beta-cyfluthrin was found to be very toxic to mammals via the oral route when administered using Cremophor as vehicle. However, Cremophor leads to unrealistically high bioavailability which does not correspond to bioavailability of beta-cyfluthrin from either formulated plant protection products or as residues on/in feed items. This is supported by a series of other studies using different vehicles such as PEG, xylene or acetone/oil. Therefore, it is concluded that all endpoints obtained excluding the data generated with Cremophor are most appropriate for risk assessment. Further, for the calculation of the geometric mean those endpoints were excluded where animals were not fasted.

Species	Rat		Mouse	
Gender	female	male	female	male
Individual LD_{50} in mg/kg bw	651	380	165	91
	336	211	-	-
	77	84	-	-
	200	-	-	-
Geomean for gender	240.9	**188.8**	165.0	**91.0**
Overall geometric mean LD_{50} in mg/kg bw (based on LD_{50} male)	**131.1**			

The overall geometric mean LD_{50} is based on the geometric mean LD_{50} derived for male rats and the LD_{50} value derived for male mice. This approach was chosen as the sensitivity to males appears to be higher than to females. The difference of the geometric means for gender (rats) is 52.1 mg/kg bw. This is 27.6 % of the lower LD_{50} (male) and 21.6 % of the higher LD_{50} (female). The difference between LD_{50} values for male and female mice is 74 mg/kg bw. This is 81.3 % of the lower LD_{50} (male) and 44.9 % of the higher LD_{50} (female). According to the EFSA GD 2009 chapter 2.1.1 the calculating of a geometric mean between the endpoints of genders should be abandoned when “the difference in the LD_{50} value is > 25 %.
The assumption of a higher sensitivity of male mammals is supported by the ADME study of [redacted] (2013b) described in Vol. 3 B6. Results show that the absorption in male animals is 1.5 fold higher than in female (in case of the highest test concentration – 10.1 mg as/kg bw). Thus, the higher sensitivity is based on a higher absorption rate of male rats.

B.9.1.2.2 Long-term and reproduction toxicity to mammals

The EU agreed NOEC for the long-term and reproduction toxcitiy to mammals is 50 ppm. This corresponds to a NOAEL of 3.3 mg/kg bw/day. According the addendum on the monograph of beta-cyfluthrin (7 May 2002) this endpoint was based on two multi-generation studies with rats [redacted] (1983), a 3- generation study and [redacted] (1996), a 2-generation study].

An expert statement was submitted arguing for a modification of the NOAEL from the multi-generation studies. The summary is presented below.

KIIA 8.13 (newly submitted with renewal dossier)

Author:	Bomann, W.
Title:	Beta-Cyfluthrin - Derivation of the relevant endpoint for use in the mammalian reproductive risk assessment for ecotoxicology
Date:	2014
Doc ID:	
Report no.:	R-34692
Edition no.:	M-483094
Guidelines:	Review, no study – no guideline
GLP:	-
Validity:	-

Abstract

Relevant studies with cyfluthrin and beta-cyfluthrin were reviewed to identify the appropriate endpoint for beta-cyfluthrin for use in the reproductive mammalian risk assessment for ecotoxicology. For this review, a combined chronic/carcinogenicity study with cyfluthrin in rats, two reproduction toxicity studies with cyfluthrin in rats and an acute, subchronic and developmental neurotoxicity study (DNT) with beta-cyfluthrinin rats were evaluated considering potential effects on mammalian populations. The evaluation resulted in a NOAEL of 17.8 mg/kg bw which was derived from the DNT study with beta-cyfluthrin. This study was regarded as the most relevant of the available studies since beta-cyfluthrin itself was tested and the study covered all parameters that are relevant for assessment of potential effects on populations.

Nevertheless, the RMS is of the opinion that the EU-agreed endpoint NOEAL of 3.3 mg/kg bw/day has to be maintained as it is still confirmed by the 2-generation study with rats [redacted] (1996)]. For details refer to Volume_3CA _B-6.6.1.

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

Substances with a high bioaccumulation potential might bear a risk of secondary poisoning for birds and mammals if feeding on contaminated prey like fish, earthworms, mammals or birds. For organic chemicals, a log P_{ow} > 3 is used to trigger an in-depth evaluation of the potential for bioaccumulation. As the log P_{ow} of the active substance beta-cyfluthrin (but not for its metabolites) is above the trigger (log P_{ow} = 5.9, see Document M-CA Section 2.7), evaluation of secondary poisoning is needed. Please refer to Volume 3CP_Bulldock_EC_ B-9.2.1 and 9.2.2 for more details.

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

The available and relevant data covering potential effects of beta-cyfluthrin on terrestrial vertebrates are presented in B.9.1.1 for birds and in B.9.1.2 for mammals.
Regarding assessment of potential effects on reptiles and amphibians neither guidance documents nor

testing guidelines are available at present.
However, other than stated by the notifier, it is possible to find information about toxic effects of pyrethroids in general as well as of cyfluthrin in particular to amphibians when searching shortly with SCOPUS. Additionally, it should have been found when using Biosis.
For example, Lambert (2001) described the death of emong tadpoles from tsetse fly control caused by the pyrethroids, cyfluthrin (40 g /ha) in Cameroon, and deltamethrin (12.5 g /ha) and permethrin (11.5 g/ha) in Burkina Faso.

Therefore, an additional extended literature concerning the toxicity of cyfluthrin and beta-cyflutrhin is required. Biosis, should be searched again. Additionally, other databases should be used, e.g. SCOPUS, PubMed. Further more the notifier should provide a justification for using the selected databases.
The notifier repeated the literature research concerning adverse effects of (beta-) cyfluthrin in scientific databases.
As a result information on toxic effects of technical beta-cyfluthrin to green frog tadpoles were submitted:

KIIA 8.1.4 (open scientitific literature)

Author:	Puglis, H.J.; Boon, M.D.
Title:	Effects of Technical-Grade Active Ingredient vs. Commercial Formulation of Seven Pesticides in the Presence or Absence of UV Radiation on Survival of Green Frog Tadpoles
Date:	2011
Doc ID:	M-479129-01-1
Report no./ DOI No.:	10.1007/s00244-010-9528-z
Source:	Arch.Environ. Contam. Toxicol., Volume 60, Issue 1, Page 145-155
Guidelines:	Review, no study – no guideline
GLP:	No. Published study (peer-review article)
Validity:	supplemental informaton

Effects of technical-grade beta-cyfluthrin and commercial formulations on the survival of *Rana clamitans* (green frog) tadpoles over 96 h under laboratory conditions were reported. NOEC or LC_{50} values were not calculated by the authors. The raw data from this experiment are not available. Thus, it is not possible to determine NOEC or LC_{50} values in a rigorous statistical way. However, the LC_{50} can be extrapolated from the graphs given in the publication. Based on mean survival values, a LC_{50} of 1.85 µg as/L (nominal) can be derived.
This value is 27 times higher than the LC_{50} for fish (0.068 µg/L in rainbow trout). Therefore, this study supports the conclusion that the fish acute endpoint derived for the as beta-cyfluthrin is also protective for amphibians.

RMS: The concluded LC_{50} of 1.85 µg as/L is not comprehensible. The lowest tested concentration was 7.5 µg/L causing more than 70 % mortality after 96 hours. The RMS did not provide a calculation of the LC_{50}.
Moreover, the mentioned concentration in tadpole test is nominal, whereas the LC_{50} in rainbow trout was derived from a flow – through test based on mean measured concentrations. Thus, these values can't be compared. When comparing this value (1.85 µg/L) or the lowest test concentration of 7.5 µg/L with the nominal LC_{50} derived from acute static laboratory tests with fish (LC_{50} = 1.06 µg as/L – 5.62 µg as/L), the toxicity of beta-cyfluthrin to fish and tadpoles are in the same range.
Therefore, an acceptable acute risk for fish might cover the acute risk for tadpoles. However, the species sensitivity distribution for effects of pyrethroids to amphibians is not known. Significant variabilities of amphibians' sensitivity were shown for the insecticide[4]. Nine *Rana* species were tested. Using

4 Bridges, C.M., Semlitsch, R.D., 2000. Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. Cons. Biol. 14, 1490–1499.

the “time-to- death-assay”, the mean time to death for green frog tadpoles (*Rana clamitans*) were approx. 18 hours. For all test species, mean time to death varied form 5 to 34 hours. It might be assumed that the variability of the sensitivity to pyrethroids is comparable.
Thus, there is uncertainty if the acute risk assement for fish covers the acute risk for amphibian tadpoles.
There is no information on chronic, sublethal effects to amphibians from public literature. Therefore, the long-term risk for amphibians remains unknown.

However, official, appropriate test guidelines for determining the chronic toxicity to amphibians are not available,
Moreover, tadpoles are only the juvenile life stage of amphibians. The adult life stage cannot be assessed or covered by the risk assessment for fish. They spend a big part of their life in terrestrial habitats, e.g. fields. Thus, they are directly exposed to the tank mixtures of pesticides.
Due to their very thin and permeable skin, contact toxicity may play a major role concerning the overall toxicity. However, little is known about the toxicity of pesticides to adult amphibian life stages.

As there are neither test guidelines for testing oral and contact toxicity to amphibian nor appropriate exposure models, the risk to amphibian cannot be addressed.

B.9.1.5 Potential for endocrine disruption

Following EU regulation 1107/2009, an assessment has to be provided concerning potential endocrine disrupting properties of the active substance concerned. Therefore, such an assessment is presented below for beta-cyfluthrin.

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

Wild Mammals

A detailed analysis of all the apical toxicological studies (developmental toxicity studies in rats and rabbits, reproductive toxicity study in rats, developmental neurotoxicity study in rats and long-term toxicity/carcinogenicity in mice and rats) on beta-cyfluthrin revealed no evidence of any reproducible endocrine effect. Therefore, based on a complete toxicological data set, there is no evidence of any endocrine disrupting potential of beta-cyfluthrin in mammals.

Birds

The population relevant effects on birds were studied in reproductive toxicity studies with cyfluthrin on bobwhite quails and mallard ducks. No statistically significant effects on adult birds, offspring or reproductive parameters were found at 269 mg cyfluthrin/kg diet in mallard ducks and 1000 mg cyfluthrin/kg diet in bobwhite quails. Reduced hatching success in both species and number of eggs laid and embryo survival in mallard duck were the most prominent effects observed in both species. No tests are currently available for birds to determine whether such findings are indeed caused by endocrine disruption or are a result of a secondary mechanism. However, since no direct endocrine disrupting potential was found in mammals it is questionable if these effects were indeed primarily triggered by an endocrine mode of action. The observed effects are not considered related to endocrine

toxicity, but a secondary consequence of general toxicity.
As there have been established levels at which reproduction was not affected in two avian species, it is concluded that based on an appropriate risk assessment, there are no population relevant adverse effects of cyfluthrin (and beta-cyfluthrin).
No further testing for endocrine disrupting properties is warranted.

B.9.2 Effects on aquatic organisms

B.9.2.1 Acute toxicity to fish

Acute studies with cold and warm water fish species are available from the data set on which the current Annex I listing of beta-cyfluthrin is based. From these data a high acute toxicity to fish was concluded in the EU review of beta-cyfluthrin with the rainbow trout as most sensitive species. The relevant endpoints of the studies are summarised in Table B.8.2-1.
In order to identify the most sensitive fish species, six additional acute tests were conducted under static conditions to reflect a single loading in the field.

Acute studies with the metabolites FPB-acid, FPB-aldehyde and DCVA are available from the data set on which the current Annex I listing of beta-cyfluthrin is based. In addition, a new acute study with *Oncorhynchus mykiss* and the metabolite FPB-acid is available and summarised below.
The metabolites FPB-acid and DCVA of beta-cyfluthrin were considered to be of no environmental concern. In the Monograph it is stated that "any harmful effects caused by these metabolites can obviously be precluded because of the data on toxicity" (Monograph, section B.7.9; Addendum 2 to Monograph, section 7.6). This conclusion for FPB-acid was confirmed by the results of a recently conducted GLP study ([redacted], 2010).

Table B.9.2-1: Acute toxicity of beta-cyfluthrin and cyfluthrin to fish

Species	Test design	LC50 (µg as/L)	NOEC (µg as/L)	Reference	reliability
Beta-Cyfluthrin					
Oncorhynchus mykiss	96 h Flowthrough (recovery 56 %)	0.089 (mm) [0.071 -0.107 µg/L]	0.053 (mm)	KIIA8.2.1/01 FF-207 [redacted], 1988 M-056119-01-2 R-19085	valid
Oncorhynchus mykiss	**96 h flowthrough**	**0.068 (mm) [0.060-0.079 µg/L]**	**< 0.039 (mm)**	**KIIA8.2.1/02 103231 [redacted], 1994 M-056053-01-1 R-19086**	**valid**
Oncorhynchus mykiss	96 h Static Recovery 33.93 %	1.06 (nom) 0.359 (mm)	0.190 (nom)	KIIA 8.2.1/07 IRV 0134/053835 [redacted], 2006a R-19596 M-481575-01-1	valid
Lepomis macrochirus	96 h flowthrough	0.280 (mm) [0,24 -0,32 µg ai/L]	0.110 (mm)	KIIA8.2.1/03 103232 [redacted], 1994 M-056058-01-1	valid
Lepomis macrochirus	96 h Static recovery 27 %	3.20 (nom) 0.870 (mm)	0.380 (nom)	KIIA 8.2.1/11 IRV 0121/053831 [redacted], 2006d	valid

				R-19596 M-482362-01-1	
Leuciscus idus melanotus	96 h flowthrough	0.331 (mm) [0,28 -0,399 µg/L]	0.190 (nom)	KIIA8.2.1/04 FO-1011 [redacted], 1988 M-056152-01-2 R-19084	valid
Gasterosteus aculeatus	96 h Static recovery 29.79 %	2.81(nom) **0.837 (mm)**	1.500 (nom)	KIIA 8.2.1/08 IRV 0123/053833 [redacted], 2006b R-19596 M-481578-01-1	valid
Rutilus rutilus	96 h Static recovery 32.4 %	1.60 (nom) **0.513 (mm)**	0.750 (nom)	KIIA 8.2.1/09 IRV 0124/053834 [redacted], 2006c R-19596 M-481577-01-1	Valid,
Pimephales promelas	96 h Static recovery 16.39 %	5.62 (nom) **1,18 (mm)**	0.750 (nom)	KIIA 8.2.1/10 IRV 0121/053831 [redacted], 2006d R-19596 M-481564-01-1	Valid,
Cyprinus carpio	96 h Static recovery 20.84 %	> 6.00 (nom) **> 1,5 (mm)**	0.380 (nom)	KIIA 8.2.1/12 IRV 0120/053830 [redacted], 2006f R-19596 M-482363-01-1	Valid,
FPB-acid					
Oncorhynchus mykiss	96 h	13000 (nom)	-	KIIA 8.2.1/14 Lit. 6002 [redacted], 1989 M-090574-01-1 R-19095	Additional information
Oncorhynchus mykiss	**96 h static**	**4060 (mm)**	**<1140 (mm)**	**KIIA 8.2.1/13 EBFRL003 [redacted], 2010 M-364414-01-1 R-27962**	**valid**
DCVA					
Oncorhynchus mykiss	**96 h static**	**>14700 (nom)**	**14700 (nom)**	**KIIA8.2.1/05 515 [redacted], 1984 M-034724-01-1 R-19097**	**valid except for missing analytical data, sub-stance is known to be stable**
FPB-aldehyde					
Oncorhynchus mykiss	**96 h static**	**792 (nom)**	**410 (nom)**	**KIIA8.2.1/06 502 [redacted], 1984 M-034806-01-1 R-19096**	**valid except for missing analytical data**

Values in bold: Endpoints used for risk assessment (tier 1)
mm: mean measured
nom: nominal initial

KIIA 8.2.1/01

Author:	[redacted]
Title:	The acute toxicity of FCR 4545 technical to rainbow trout (Salmo gairdneri, Richardson) in a flow-through test
Date:	1988
Doc ID:	M-056119-01-2
Report no.:	FF-207
Guidelines:	EEC 79/831 Method V C.I, OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, § 72-1.
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-cyfluthrin techn. (FCR 4545), purity: 98.1 %, batch no. 16001/87
Results: The LC_{50} (96h) of beta-cyfluthrin technical (FCR 4545) was 0.089 µg as/L with a 95 % confidence interval of LC_{50} (96h) 0.071 – 0.107 µg as/L (based on mean measured concentrations). The lowest lethal concentration (LLC) was 0.078 µg as/L. The no observed effect concentration (NOEC) was 0.053 µg as/L.

Conclusion: LC_{50} (96h, flow-through) = 0.089 µg as/L

KIIA 8.2.1/02

Author:	[redacted]
Title:	Acute toxicity of FCR 4545 technical to rainbow trout (Oncorhynchus mykiss) under flow-through conditions
Date:	1994
Doc ID:	M-056053-01-1
Report no.:	103231
Guidelines:	US-EPA FIFRA § 72-1 guideline
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-Cyfluthrin techn. (FCR 4545), purity: 99.4 %, batch no. 88R0256I
Results: The 96h LC_{50} value of beta-cyfluthrin technical was 0.068 µg as/L with a 95 % confidence interval of 0.060 – 0.079 µg as/L (based on mean measured concentrations). The no observed effect concentration (NOEC) was < 0.039 µg as/L.

Conclusion: LC_{50} (96 h, flow-through) = 0.068 µg as/L

KIIA 8.2.1/03

Author:	[redacted]
Title:	Acute toxicity of FCR 4545 technical to bluegill (*Lepomis macrochirus*) under flow-through conditions
Date:	1994
Doc ID:	M-056058-01-1
Report no.:	103232
Guidelines:	US-EPA FIFRA § 72-1 guideline
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-cyfluthrin techn. (FCR 4545), purity: 99.4 %, batch no. 88R0256I
Results: The 96h LC_{50} value of beta-cyfluthrin technical was 0.28 µg as/L with a 95 % confidence interval of 0.24 – 0.32 µg as/L (based on mean measured concentrations). The no observed effect concentration (NOEC) was 0.11 µg as/L.

Conclusion: LC_{50} (96 h, flow-through) = 0.28 µg as/L

KIIA 8.2.1/04

Author:	[redacted]
Title:	The acute toxicity of FCR 4545 technical to golden orfe (*Leuciscus idus melanotus*) in a flow-through test
Date:	31 May 1988
Doc ID:	M-056152-01-2
Report no.:	FO-1011
Guidelines:	EEC 79/831 Method V C.I, OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, § 72-1
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203
Material: beta-cyfluthrin techn. (FCR 4545), purity: 98.1 %, batch no. 16001/87
Results: The 96h LC_{50} (of beta-cyfluthrin technical was 0.331 µg as/L with a 95 % confidence interval of 0.2801 – 0.3987 µg as/L (based on mean measured concentrations). The lowest lethal concentration (LLC) was 0.496 µg as/L. The no observed effect concentration (NOEC) was 0.1988 µg as/L.

Conclusion: LC_{50} (96 h, flow-through) = 0.331 µg as/L

KIIA 8.2.1/07 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Beta-Cyfluthrin: acute toxicity to rainbow trout
Date:	10 March 2006a
Doc ID:	M-481575-01-1
Report no.:	IRV0134/053835
Guidelines:	EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Deviations: As the measured concentration of the substance decreased, with 22 to 33 % of nominal at 48 hours and between 19 and 21 % of nominal at 96 hours, endpoints based on nominal values are not appropriate. Therefore, endpoints based on mean measured values were calculated by the RMS. Other validity criteria according to the current OECD Guideline No. 203 are fulfilled.

Executive Summary
The acute effects of beta-cyfluthrin to the rainbow trout (*Oncorhynchus mykiss*) were investigated under static conditions. This exposure regime was selected in order to be relevant to a single exposure loading in the field.
Groups of seven juvenile fish were exposed to beta-cyfluthrin technical, dispersed in water at nominal concentrations of 0.19, 0.38, 0.75, 1.5 and 3.0 µg/L. One untreated control and one additional control group containing dimethylformamide and water (100 µL/L) were included in the study. Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure.

At the start of the test, the measured concentrations ranged between 70 and 94 % of their nominal concentrations. Thereafter, the measured values decreased, with 22 to 33 % of nominal at 48 hours and between 19 and 21 % of nominal at 96 hours. The average recovery rate was 33.93 %.
Based on nominal concentrations, the 96-hour LC50 is 1.06 µg/L beta-cyfluthrin (95 % C.L. 0.75-1.5 µg/L) and the NOEC is 0.19 µg/L. Based on mean measured concentrations the 96-hour LC50 is 0.359 µg/L beta-cyfluthrin and the NOEC is 0.068 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Description:	Powder
Lot/Batch #:	030916
Purity:	96.1 %

2. Vehicle and/or positive control: None

3. Test organism:

Species:	Rainbow trout (Oncorhynchus mykiss)
Age:	Juvenile
Size:	mean fork length: 5.7 cm
Body weight of the animals:	mean wet weight: 2.34 g
Loading:	initial static loading of 0.74 g body weight/L
Source:	Commercial fish farm in the UK
Culture:	Stock of fish obtained from supplier on 25 August 2005 and held in aerated supply of diluent water under flow-through conditions
Diet/Food:	Standard commercial fish food (TROUW (UK) Ltd; Nutra Fry 02) in an amount equivalent to 1.0-1.5 % of the total wet-weight of fish in the holding tank. Food was supplied daily until 26 hours prior to testing.
Acclimation period:	14 days

4. Environmental conditions:

Temperature:	Control (min-max): 15.2-15.3 solvent control (min-max): 15.0-15.4 0.38 µg/L (min-max): 15.0-15.4 0.75 µg/L (min-max): 145.0-15.3 1.5 µg/L (min-max): 15.1-15.3 3.0 µg/L(min-max): 15.2-15.2 6.0 µg/L (min-max): 15.2-15.8
Photoperiod:	16 hours light, 8 hours dark
pH:	Control (min-max): 7.72-8.11 solvent control (min-max): 7.88-8.03 0.38 µg/L (min-max): 7.87-8.21 0.75 µg/L (min-max): 7.89-8.16 1.5 µg/L (min-max): 8.00-8.29 3.0 µg/L(min-max): 8.00-8.03 6.0 µg/L (min-max): 8.03-8.29
Dissolved oxygen [% air saturation value]:	Control (min-max): 83-103 solvent control (min-max): 82-101 0.38 µg/L (min-max): 86-103 0.75 µg/L (min-max): 88-104

1.5 µg/L (min-max): 92-105
3.0 µg/L(min-max): 100-104
6.0 µg/L (min-max): 101-104
Conductivity: 350 µS/cm³ in the dilution water at 20 °C
Total hardness: 172 mg/L as $CaCO_3$

B. STUDY DESIGN

1. Experimental conditions

A range finding test was followed by a definitive test with five test concentrations. Control groupswere exposed to diluent water alone and diluent water plus auxiliary solvent (100 µL dimethylformamide/L). The definitive test was conducted at nominal beta-cyfluthrin technical concentrations of 0.19, 0.38, 0.75, 1.5 and 3.0 µg/L. In the definitive test, groups of seven fish were placed at random into each glass aquarium containing the prepared control or test media (fish were added at the same time as the test substance). Each vessel contained 22 litres of medium to a depth of 22 cm. This provided an initial static loading of 0.74 g body weight/L. The fish were exposed to the control or test conditions for a period of 96 hours under static conditions, with no renewal of media.
Treatment and control groups were maintained at 15 ± 2 °C throughout the exposure period andconstant to within ± 1 °C during the study. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light and 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The fish were not fed during the 96-hour exposure period.

2. Observations

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle. In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish. Daily records of temperature, pH and dissolved oxygen were kept performed for each control and test vessel. Total hardness was measured at 0 hours in the control and the highest test concentration.

3. Statistical calculations

The LC_{50} was calculated using the SAFEStat LD_{50} application (SAS 8.2.) and the nominal concentrations. The NOEC was derived by direct inspection of the data for lethal and treatment related effects. An incidence rate of more than one affected fish out of seven is considered to be significant.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

Analytical data: The measured concentrations of beta-cyfluthrin ranged between 70 and 94 % of their nominal values in samples of freshly prepared media. Thereafter, the measured concentrations decreased with between 22 and 33 % of their nominal values at 48 hours and between 19 and 21 % of the nominal concentrations at 96 hours. A low but measurable concentration of beta-cyfluthrin (0.06 µg/L) was found in the samples taken from the solvent control vessel on day 0. Since no adverse effects were noted in the solvent control vessel, the validity of the test was not considered to have been affected.

The 96-hour LC_{50} and NOEC values are presented below. The test results are expressed in terms of the nominal concentrations of beta-cyfluthrin technical.

Endpoints	Beta-cyfluthrin technical [µg/L]
LC_{50} (95 % C.L.) (96 h)	1.06 (0.75-1.5)
NOEC (96 h)	0.19

B. OBSERVATIONS

Treatment effects occurred at 0.38 µg/L and higher concentrations and comprised effects on behaviour, pigmentation, respiration and co-ordination. At these concentrations, the symptoms were exhibited within the initial two hours of exposure but they were not sustained and from 48 hours onwards, all of the surviving fish appeared to have recovered and were normal.

At the two highest nominal concentrations employed in the test (1.5 and 3.0 µg/L), 100 % mortality occurred. The effects on mortality of beta-cyfluthrin in the rainbow trout are summarised below.

Beta-cyfluthrin (µg/L, nom.)	Cumulative mortality (initial population = 7 fish/concentration)						
	2h	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0
0.19	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0
0.75	0	0	0	0	0	0	0
1.5	0	0	7	7	7	7	100
3.0	4	7	7	7	7	7	100

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) remained within acceptable limits throughout the study.

III. CONCLUSION

The 96-hour LC_{50} for the rainbow trout (*Oncorhynchus mykiss*) exposed to beta-cyfluthrin technical under static conditions is 1.06 µg/L (nominal; 95 % C.L. 0.75-1.5 µg/L). The NOEC (96 h) is 0.19 µg/L beta-cyfluthrin technical.
Based on mean measured concentrations the 96-hour LC_{50} is 0.359 µg/L beta-cyfluthrin and the NOEC is 0.068 µg/L.

KIIA 8.2.1/08 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Beta-Cyfluthrin – acute toxicity to three-spined stickleback
Date:	10 March 2006b
Doc ID:	M-481578-01-1
Report no.:	IRV0123/053833
Guidelines:	EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Guideline: EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
Deviations: As the measured concentration of the substance decreased, with 22 to 38 % of nominal at 48 hours and between 12 and 18 % of nominal at 96 hours, endpoints based on nominal values are not appropriate. Therefore, endpoints based on mean measured values were calculated by the RMS. Other validity criteria according to the current OECD Guideline No. 203 are fulfilled.

Dates of experimental work: 21 July 2005 to 18 November 2005
Executive Summary
The acute effects of beta-cyfluthrin to the three-spined stickleback (Gasterosteus aculeatus) were investigated under static conditions. This exposure regime was selected in order to be relevant to a single exposure loading in the field.
Groups of seven juvenile fish were exposed to beta-cyfluthrin technical, dispersed in water at nominal

concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. One untreated control and one additional control group containing dimethylformamide and water (100 µL/L) were included in the study. Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure.

At the start of the test, the measured concentrations ranged between 71 and 91 % of their nominal concentrations. Thereafter, the measured values decreased, with 22 to 38 % of nominal at 48 hours and between 12 and 18 % of nominal at 96 hours The average recovery rate was 29.79 %.

Based on nominal concentrations, the 96-hour LC50 is 2.81 µg/L beta-cyfluthrin (95 % C.L. 2.26-3.62 µg/L) and the NOEC is 1.5 µg/L. Based on mean measured concentrations the 96-hour LC_{50} is 0.837 µg/L beta-cyfluthrin and the NOEC is 0.447 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Description:	Powder
Lot/Batch #:	030916
Purity:	96.1 %

2. Vehicle and/or positive control: None

3. Test organism:

Species:	Three-spined stickleback (*Gasterosteus aculeatus*)
Age:	Juvenile
Size:	mean fork length: 4.3 cm
Body weight of the animals:	mean wet weight: 1.0 g
Loading:	initial static loading of 0.64 g body weight/L
Source:	[redacted]
Culture:	Stock of fish obtained from supplier on 26 August 2005 and held in aerated supply of diluent water under flow-through conditions
Diet/Food:	Standard commercial fish food (TROUW (UK) Ltd; Nutra Fry 02) in an amount equivalent to 1.5-2.0 % of the total wet-weight of fish in the holding tank and supplemented with bloodworms. Food was supplied daily until 27 hours prior to testing.
Acclimation period:	14 days

4. Environmental conditions:

Temperature:	Control (min-max): 14.9-15.7 solvent control (min-max): 14.9-15.8 0.38 µg/L (min-max): 14.8-15.6 0.75 µg/L (min-max): 14.8-15.6 1.5 µg/L (min-max): 14.8-15.6 3.0 µg/L(min-max): 15.0-15.6 6.0 µg/L (min-max): 15.1-15.6
Photoperiod:	16 hours light, 8 hours dark
pH:	Control (min-max): 7.71-8.17 solvent control (min-max): 7.80-8.26 0.38 µg/L (min-max): 7.82-8.26 0.75 µg/L (min-max): 7.85-8.36 1.5 µg/L (min-max): 7.86-8.44 3.0 µg/L(min-max): 7.88-8.50 6.0 µg/L (min-max): 7.88-8.01

Dissolved oxygen [% air saturation value]:	Control (min-max): 94-105 solvent control (min-max): 94-104 0.38 µg/L (min-max): 85-102 0.75 µg/L (min-max): 93-106 1.5 µg/L (min-max): 93-108 3.0 µg/L(min-max): 94-111 6.0 µg/L (min-max): 82-103
Conductivity:	350 µS/cm³ in the dilution water at 20 °C
Total hardness:	164-176 mg/L as CaCO3

B. STUDY DESIGN AND METHODS

1. Experimental treatments

A range finding test was followed by a definitive test with five test concentrations. Control groups were exposed to diluent water alone and diluent water plus auxiliary solvent (100 µL dimethylformamide/L). The definitive test was conducted at nominal beta-cyfluthrin technical concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. In the definitive test, groups of seven fish were placed at random into each glass aquarium containing the prepared control or test media (fish were added at the same time as the test substance). Each vessel contained 11 litres of medium to a depth of 16.5 cm. This provided an initial static loading of 0.64 g body weight/L. The fish were exposed to the control or test conditions for a period of 96 hours under static conditions, with no renewal of media.
Treatment and control groups were maintained at 15 ± 2 °C throughout the exposure period and constant to within ± 1 °C during the study. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light and 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The fish were not fed during the 96-hour exposure period.

2. Observations

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle. In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish. Daily records of temperature, pH and dissolved oxygen were kept performed for each control and test vessel. Total hardness was measured at 0 hours in the control and the highest test concentration.

3. Statistical calculations

The LC_{50} was calculated using the SAFEStat LD50 application (SAS 8.2.) and the nominal concentrations. The NOEC was derived by direct inspection of the data for lethal and treatment-related effects. An incidence rate of more than one affected fish out of seven is considered to be significant.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

Analytical data: The measured concentrations of beta-cyfluthrin ranged between 71 and 91 % of their nominal values in samples of freshly prepared media. Thereafter, the measured concentrations decreased, with between 22 and 38 % of their nominal values at 48 hours and between 12 and 18 % of nominal at 96 hours.
The 96-hour LC5 0and NOEC values are presented below. The test results are expressed in terms of the nominal concentrations of beta-cyfluthrin technical.

Endpoints	Beta-Cyfluthrin technical [µg/L]
LC50 (95 % C.L.) (96 h)	2.81 (2.26-3.62)
NOEC (96 h)	1.5

B. OBSERVATIONS

Treatment effects occurred at 3.0 µg/L and comprised effects on pigmentation, respiration and coordination.
At 6.0 µg/L, symptoms were exhibited within the initial fifteen minutes of exposure and all fish had died within 48 hours. At 3 µg/L, all fish exhibited adverse effects within the initial two hours of exposure and four fish died during the initial 48 hours; between 48 and 96 hours, one of the three surviving fish appeared to have recovered and was normal at 72 and 96 hours.

At the highest nominal concentration employed in the test (6.0 µg/L), 100 % mortality occurred. The effects on mortality of beta-cyfluthrin in the three-spined stickleback are summarised below.

Table B.9.2-2: Effects on mortality of beta-cyfluthrin in the three-spined stickleback

Beta-Cyfluthrin (µg/L, nom.)	Cumulative mortality (initial population = 7 fish/concentration)						
	2h	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0
0.75	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0
3.0	0	0	1	4	4	4	57
6.0	0	0	5	7	7	7	100

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) remained within acceptable limits throughout the study.

III. CONCLUSION

The 96-hour LC_{50} for the three-spined stickleback (*Gasterosteus aculeatus*) exposed to beta-cyfluthrin technical under static conditions is 2.81 µg/L (nominal; 95 % C.L. 2.26-3.62 µg/L). The NOEC after 96 h is 1.5 µg/L beta-cyfluthrin technical. Based on mean measured concentrations the 96-hour LC_{50} is 0.837 µg/L beta-cyfluthrin and the NOEC is 0.447 µg/L.

KIIA 8.2.1/09 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Beta-Cyfluthrin – acute toxicity to roach
Date:	10 March 2006c
Doc ID:	
Report no.:	IRV0124/053834
Edition no.:	M-481577-01-1 (R-19596)
Guidelines:	EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Guideline: EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
Deviations: As the measured concentration of the substance decreased, with 17 to 29 % of nominal at 48 hours and between 18 and 19 % of nominal at 96 hours, endpoints based on nominal values are not

appropriate. Therefore, endpoints based on mean measured values were calculated by the RMS. Other validity criteria according to the current OECD Guideline No. 203 are fulfilled.

Dates of experimental work: 22 August 2005 to 18 November 2005

Executive Summary

The acute effects of beta-cyfluthrin to the roach (*Rutilus rutilus*) were investigated under static conditions. This exposure regime was selected in order to be relevant to a single exposure loading in the field. Groups of seven juvenile fish were exposed to beta-cyfluthrin technical, dispersed in water at nominal concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. One untreated control and one additional control group containing dimethylformamide and water (100 µL/L) were included in the study. Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure.
At the start of the test, the measured concentrations ranged between 70 and 84 % of their nominal concentrations. Thereafter, the measured values decreased, with 17 to 29 % of nominal at 48 hours and between 18 and 19 % of nominal at 96 hours. The average recovery rate was 32.04 %.

Based on nominal concentrations, the 96-hour LC_{50} is 1.6 µg/L beta-cyfluthrin (95 % C.L. 1.24-1.99 µg/L) and the NOEC is 0.75 µg/L. Based on mean measured concentrations the 96-hour LC_{50} is 0.513 µg/L beta-cyfluthrin and the NOEC is 0.240 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Description:	Powder
Lot/Batch #:	030916
Purity:	96.1 %
2. Vehicle and/or positive control:	None
3. Test organism:	
Species:	Roach (*Rutilus rutilus*)
Age:	Juvenile
Size:	mean fork length: 4.5 cm
Body weight of the animals:	mean wet weight: 1.29 g
Loading:	initial static loading of 0.7 g body weight/L
Source:	Commercial fish farm in the UK
Culture:	Stock of fish obtained from supplier on 22 July 2005 and held in aerated supply of diluent water under flow-through conditions
Diet/Food:	Standard commercial fish food (TROUW (UK) Ltd; Nutra Fry 02) in an amount equivalent to 1.5-2.0 % of the total wet-weight of fish in the holding tank and supplemented with bloodworms. Food was supplied daily until 27 hours prior to testing.
Acclimation period:	14 days

4. Environmental conditions:

Temperature:	Control (min-max): 15.0-15.4 solvent control (min-max): 15.0-15.4 0.38 µg/L (min-max): 14.9-15.3 0.75 µg/L (min-max): 14.9-15.3 1.5 µg/L (min-max): 15.0-15.2 3.0 µg/L(min-max): 15.1-15.3 6.0 µg/L (min-max): 15.1-15.2
Photoperiod:	16 hours light, 8 hours dark

pH:	Control (min-max): 7.83-8.25 solvent control (min-max): 7.93-8.27 0.38 µg/L (min-max): 7.95-8.37 0.75 µg/L (min-max): 7.95-8.40 1.5 µg/L (min-max): 7.94-8.46 3.0 µg/L(min-max): 7.93-8.01 6.0 µg/L (min-max): 7.95-8.28
Dissolved oxygen [% air saturation value]:	Control (min-max): 89-105 solvent control (min-max): 96-106 0.38 µg/L (min-max): 99-108 0.75 µg/L (min-max): 100-108 1.5 µg/L (min-max): 98-108 3.0 µg/L(min-max): 100-105 6.0 µg/L (min-max): 102-105
Conductivity:	350 µS/cm³ in the dilution water at 20 °C
Total hardness:	170-172 mg/L as CaCO3

B. STUDY DESIGN AND METHODS

1. Experimental treatments

A range finding test was followed by a definitive test with five test concentrations. Control groups were exposed to diluent water alone and diluent water plus auxiliary solvent (100 µL dimethylformamide/L). The definitive test was conducted at nominal beta-cyfluthrin technical concentrationsof 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. In the definitive test, groups of seven fish were placed atrandom into each glass aquarium containing the prepared control or test media (fish were added at the same time as the test substance). Each vessel contained 13 litres of medium to a depth of 17.5 cm. This provided an initial static loading of 0.7 g body weight/L. The fish were exposed to the control or test conditions for a period of 96 hours under static conditions, with no renewal of media.
Treatment and control groups were maintained at 15 ± 2 °C throughout the exposure period and constant to within ± 1 °C during the study. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light and 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The fish were not fed during the 96-hour exposure period.

2. Observations

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle. In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish. Daily records of temperature, pH and dissolved oxygen were kept performed for each control and test vessel. Total hardness was measured at 0 hours in the control and the highest test concentration.

3. Statistical calculations

The LC_{50} was calculated using the SAFEStat LD50 application (SAS 8.2.) and the nominal concentrations. The NOEC was derived by direct inspection of the data for lethal and treatmen-trelated effects. An incidence rate of more than one affected fish out of seven is considered to be significant.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

Analytical data: The measured concentrations of beta-cyfluthrin ranged between 70 and 84 % of their nominal values in samples of freshly prepared media. Thereafter, the measured concentrations decreased, with between 17 and 29 % of their nominal values at 48 hours and between 18 and 19 % of

nominal at 96 hours.
The 96-hour LC_{50} and NOEC values are presented below. The test results are expressed in terms of the nominal concentrations of beta-cyfluthrin technical.

Endpoints	Beta-Cyfluthrin technical [µg/L]
LC_{50} (95 % C.L.) (96 h)	1.6 (1.24-1.99)
NOEC (96 h)	0.75

B. OBSERVATIONS
Treatment effects occurred at 1.5 µg/L and higher concentrations and comprised effects on pigmentation, respiration and co-ordination. At these exposure levels, the symptoms were exhibited within the initial two hours of exposure. At 3 and 6 µg/L, all of the fish were dead at 24 hours. At 1.5 µg/L, three fish died during the initial 48 hours of exposure but two of the surviving fish appeared to have recovered and were normal at 72 and 96 hours.
At the two highest nominal concentrations employed in the test (3.0 and 6.0 µg/L), 100 % mortality occurred. The effects on mortality of beta-cyfluthrin in the roach are summarised below.

Table B.9.2-3: Effects on mortality of beta-cyfluthrin in the roach

Beta-Cyfluthrin (µg/L, nom.)	Cumulative mortality (initial population = 7 fish/concentration)						
	2h	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0
0.75	0	0	0	0	0	0	0
1.5	0	1	2	3	3	3	43
3.0	0	0	7	7	7	7	100
6.0	0	0	7	7	7	7	100

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) remained within acceptable limits throughout the study.

III. CONCLUSION

The 96-hour LC_{50} for the roach (*Rutilus rutilus*) exposed to beta-cyfluthrin technical under static conditions is 1.6 µg/L (nominal; 95 % C.L. 1.24-1.99 µg/L). The NOEC (96 h) is 0.75 µg/L beta-cyfluthrin technical. Based on mean measured concentrations the 96-hour LC_{50} is 0.513 µg/L beta-cyfluthrin and the NOEC is 0.240 µg/L.

KIIA 8.2.1/10 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Beta-Cyfluthrin – acute toxicity to fathead minnow
Date:	10 March 2006d
Doc ID:	M-481564-01-1
Report no.:	IRV0121/053831
Guidelines:	EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Deviations: As the measured concentration of the substance decreased, with 5 to 19 % of nominal at 48 hours and between 3 and 5 % of nominal at 96 hours, endpoints based on nominal values are not appropriate. Therefore, endpoints based on mean measured values were calculated by the RMS. Other validity criteria according to the current OECD Guideline No. 203 are fulfilled.
Dates of experimental work: 21 July 2005 to 1 December 2005

Executive Summary
The acute effects of beta-cyfluthrin to fathead minnow (Pimephales promelas) were investigated under static conditions. This exposure regime was selected in order to be relevant to a single exposure loading in the field.
Groups of seven juvenile fish were exposed to beta-cyfluthrin technical, dispersed in water at nominal concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. One untreated control and one additional control group containing dimethylformamide and water (100 µL/L) were included in the study. Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure.
At the start of the test, the measured levels ranged between 95 and 100 % of nominal for four of the treatment concentrations. For the 0.75 µg/L group, the measured value was 151 % of the nominal concentration. Thereafter, the measured concentrations decreased, with 5 to 19 % of nominal at 48 hours and between 3 and 5 % of nominal at 96 hours. The average recovery rate was 16.39 %.

Based on nominal concentrations, the 96-hour LC_{50} is 5.62 µg/L beta-cyfluthrin (95 % C.L. 4.49-8.92 µg/L) and the NOEC is 0.75 µg/L. Based on mean measured concentrations the 96-hour LC_{50} is 1.18 µg/L beta-cyfluthrin and the NOEC is 0.123 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Description:	Powder
Lot/Batch #:	030916
Purity:	96.1 %
2. Vehicle and/or positive control:	None
3. Test organism:	
Species:	Fathead minnow (*Pimephales promelas*)
Age:	Juvenile
Size:	mean fork length: 2.4 cm
Body weight of the animals:	mean wet weight: 0.53 g
Loading:	initial static loading of 0.4 g body weight/L
Source:	[redacted]
Culture:	Stock of fish obtained from supplier on 27 October 2005 and held in recirculated supply of diluent water
Diet/Food:	Standard commercial fish food (TetraMin Complete Flake Food) in an amount equivalent to 4 % of the total wet-weight of fish in the holding tank and supplemented with *Artemia* and bloodworms. Food was supplied daily until 23 hours prior to testing.
Acclimation period:	14 days

4. Environmental conditions:

Temperature:	Control (min-max): 21.3-22.9 solvent control (min-max): 21.4-22.9 0.38 µg/L (min-max): 21.4-23.0 0.75 µg/L (min-max): 21.6-22.9 1.5 µg/L (min-max): 21.5-22.9 3.0 µg/L(min-max): 21.8-22.8 6.0 µg/L (min-max): 21.6-22.9
Photoperiod:	16 hours light, 8 hours dark

pH:	Control (min-max): 7.81-8.55 solvent control (min-max): 7.99-8.49 0.38 µg/L (min-max): 8.19-8.51 0.75 µg/L (min-max): 8.23-8.49 1.5 µg/L (min-max): 8.27-8.52 3.0 µg/L(min-max): 8.30-8.53 6.0 µg/L (min-max): 8.35-8.59
Dissolved oxygen [% air saturation value]:	Control (min-max): 93-106 solvent control (min-max): 93-108 0.38 µg/L (min-max): 92-109 0.75 µg/L (min-max): 91-106 1.5 µg/L (min-max): 90-107 3.0 µg/L(min-max): 91-109 6.0 µg/L (min-max): 94-110
Conductivity:	350 µS/cm³ in the dilution water at 20 °C
Total hardness:	180-182 mg/L as CaCO3

B. STUDY DESIGN AND METHODS

1. Experimental treatments

A range finding test was followed by a definitive test with five test concentrations. Control groups were exposed to diluent water alone and diluent water plus auxiliary solvent (100 µL dimethylformamide/L). The definitive test was conducted at nominal beta-cyfluthrin technical concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. In the definitive test, groups of seven fish were placed atrandom into each glass aquarium containing the prepared control or test media (fish were added at the same time as the test substance). Each vessel contained 10 litres of medium to a depth of 16.5 cm. This provided an initial static loading of 0.4 g body weight/L. The fish were exposed to the control or test conditions for a period of 96 hours under static conditions, with no renewal of media. Treatment and control groups were maintained at 22 ± 2 °C throughout the exposure period and constant to within ± 1 °C during the study. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light: 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The fish were not fed during the 96 hour exposure period.

2. Observations

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle. In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish. Daily records of temperature, pH and dissolved oxygen were kept performed for each control and test vessel. Total hardness was measured at 0 hours in the control and the highest test concentration.

3. Statistical calculations

The LC_{50} was calculated using the SAFEStat LD50 application (SAS 8.2.) and the nominal concentrations. The NOEC was derived by direct inspection of the data for lethal and treatment-related effects. An incidence rate of more than one affected fish out of seven is considered to be significant.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

Analytical data: The measured concentrations of beta-cyfluthrin ranged between 95 and 151 % of their nominal values in samples of freshly prepared media. Thereafter, the measured concentrations de-

creased,with between 5 and 19 % of their nominal values at 48 hours and between 3 and 5 % of nominal at 96 hours.
Measurable levels of beta-cyfluthrin (0.253 to 0.290 µg/L) were found in the samples taken from the diluent water and solvent control vessels on day 0 and thereafter, in samples taken at 24 and 48 hours, with between 0.0928 and 0.0980 µg/L remaining at 48 hours. At 96 hours, the measured levels of betacyfluthrin in control samples were below the limit of quantification of the analytical method (0.02 µg/L). Although the cause of this contamination was not identified, the vessels used for the control groups were thought to have been the source of the test substance as they had been used previously in other studies performed on the same test substance. Since no adverse effects were noted in either of the control vessels or at the two lowest test levels, the validity of the test was not thought to have been affected.

The 96-hour LC_{50} and NOEC values are presented below. The test results are expressed in terms of the nominal concentrations of beta-cyfluthrin technical.

Endpoints	**Beta-Cyfluthrin technical [µg/L]**
LC_{50} (95 % C.L.) (96 h)	5.62 (4.49-8.92)
NOEC (96 h)	0.75

B. OBSERVATIONS
Treatment effects were observed at 1.5 µg/L and higher concentrations and comprised effects on behaviour, pigmentation, respiration and co-ordination. At these exposure levels, the symptoms were exhibited within the initial two hours of exposure. At 3.0 and 6.0 µg/L, the fish were adversely affected throughout the test although the symptoms exhibited at 96 hours were not as severe as those noted during the initial 24 hours of exposure. At 1.5 µg/L, the fish appeared to have recovered at 48 hours and were normal for the remainder of the test.
At the highest nominal concentration (6.0 µg/L) employed in the test, 57 % mortality occurred. The effects on mortality of beta-cyfluthrin in the fathead minnow are summarised below.

Table B.9.2-4: Effects on mortality of beta-cyfluthrin in the fathead minnow

Beta-Cyfluthrin (µg/L, nom.)	**Cumulative mortality (initial population = 7 fish/concentration)**						
	2h	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0
0.75	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0
3.0	0	0	0	0	0	0	0
6.0	0	0	4	4	4	4	57

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) remained within acceptable limits throughout the study.

III. CONCLUSION

The 96-hour LC_{50} for the fathead minnow (*Pimephales promelas*) exposed to beta-cyfluthrin technical under static conditions is 5.62 µg/L (nominal; 95 % C.L. 4.49-8.92 µg/L). The NOEC (96 h) is 0.75 µg/L beta-cyfluthrin technical. Based on mean measured concentrations the 96-hour LC_{50} is 1.18 µg/L beta-cyfluthrin and the NOEC is 0.123 µg/L.

KIIA 8.2.1/11 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Beta-Cyfluthrin – acute toxicity to bluegill sunfish
Date:	10 March 2006e
Doc ID:	M-482362-01-1
Report no.:	IRV0122/053832
Guidelines:	EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Deviations: As the measured concentration of the substance decreased, 14 and 23 % of their nominal values at 48 hours and between 8 and 15 % of nominal at 96 hours, endpoints based on nominal values are not appropriate. Therefore, endpoints based on mean measured values were calculated by the RMS. Other validity criteria according to the current OECD Guideline No. 203 are fulfilled.

Dates of experimental work: 5 July 2005 to 17 November 2005

Executive Summary

The acute effects of beta-cyfluthrin to bluegill sunfish (*Lepomis macrochirus*) were investigated under static conditions. This exposure regime was selected in order to be relevant to a single exposure loading in the field.
Groups of seven juvenile fish were exposed to beta-cyfluthrin technical, dispersed in water at nominal concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. One untreated control and one additional control group containing dimethylformamide and water (100 µL/L) were included in the study. Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure.
At the start of the test, the measured concentrations ranged between 92 and 130 % of their nominal concentrations. Thereafter, the measured values decreased, with 14 to 23 % of nominal at 48 hours and between 8 and 15 % of nominal at 96 hours. The average recovery rate was 27 %.

Based on nominal concentrations, the 96-hour LC_{50} is 3.2 µg/L beta-cyfluthrin (95 % C.L. 2.49-4.0 µg/L) and the NOEC is 0.38 µg/L. Based on mean measured concentrations the 96-hour LC_{50} is 0.87 µg/L beta-cyfluthrin and the NOEC is 0.103 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Beta-cyfluthrin technical
Description: Powder
Lot/Batch #: 030916
Purity: 96.1 %

2. Vehicle and/or positive control: None

3. Test organism:

Species: Bluegill sunfish (Lepomis macrochirus)
Age: Juvenile
Size: mean fork length: 3.9 cm
Body weight of
the animals: mean wet weight: 1.27 g
Loading: initial static loading of 0.68 g body weight/L

Source:	[redacted]
Culture:	Stock of fish obtained from supplier on 26 May 2005 and held in recirculated supply of diluent water
Diet/Food:	Standard commercial fish food (TetraPro Flake Food) in an amount equivalent to 1-2 % of the total wet-weight of fish in the holding tank and supplemented with Artemia and bloodworms. Food was supplied daily until 22 hours prior to testing.
Acclimation period:	14 days

4. Environmental conditions:

Temperature:	Control (min-max): 21.4-22.6 solvent control (min-max): 21.7-22.6 0.38 µg/L (min-max): 21.8-22.5 0.75 µg/L (min-max): 21.8-22.6 1.5 µg/L (min-max): 21.8-22.7 3.0 µg/L(min-max): 21.8-22.7 6.0 µg/L (min-max): 21.8-22.9
Photoperiod:	16 hours light, 8 hours dark
pH:	Control (min-max): 7.97-8.40 solvent control (min-max): 7.95-8.46 0.38 µg/L (min-max): 7.99-8.54 0.75 µg/L (min-max): 8.01-8.47 1.5 µg/L (min-max): 8.03-8.49 3.0 µg/L(min-max): 8.10-8.62 6.0 µg/L (min-max): 8.11-8.11
Dissolved oxygen [% air saturation value]:	Control (min-max): 87-104 solvent control (min-max): 93-102 0.38 µg/L (min-max): 90-104 0.75 µg/L (min-max): 92-103 1.5 µg/L (min-max): 89-102 3.0 µg/L(min-max): 83-101 6.0 µg/L (min-max): 92-103
Conductivity:	350 µS/cm³ in the dilution water at 20 °C
Total hardness:	166-180 mg/L as CaCO3

B. STUDY DESIGN AND METHODS

1. Experimental treatments

A range finding test was followed by a definitive test with five test concentrations. Control groups were exposed to diluent water alone and diluent water plus auxiliary solvent (100 µL dimethylformamide/L). The definitive test was conducted at nominal beta-cyfluthrin technical concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. In the definitive test, groups of seven fish were placed at random into each glass aquarium containing the prepared control or test media (fish were added at the same time as the test substance). Each vessel contained 13 litres of medium to a depth of 17.5 cm. This provided an initial static loading of 0.68 g body weight/L. The fish were exposed to the control or test conditions for a period of 96 hours under static conditions, with no renewal of media. Treatment and control groups were maintained at 22 ± 2 °C throughout the exposure period and constant to within ± 1 °C during the study. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light and 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The fish were not fed during the 96-hour exposure period.

2. Observations

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence

of response to physical stimulation of the caudal peduncle. In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish. Daily records of temperature, pH and dissolved oxygen were kept performed for each control and test vessel. Total hardness was measured at 0 hours in the control and the highest test concentration.

3. Statistical calculations
The LC_{50} was calculated using the SAFEStat LD_{50} application (SAS 8.2.) and the nominal concentrations. The NOEC was derived by direct inspection of the data for lethal and treatment-related effects. An incidence rate of more than one affected fish out of seven is considered to be significant.

II. RESULTS AND DISCUSSION

A. FINDINGS
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.
Analytical data: The measured concentrations of beta-cyfluthrin ranged between 92 and 130 % of their nominal values in samples of freshly prepared media. Thereafter, the measured concentrations decreased, with between 14 and 23 % of their nominal values at 48 hours and between 8 and 15 % of nominal at 96 hours.
The 96-hour LC_{50} and NOEC values are presented below. The test results are expressed in terms of the nominal concentrations of beta-cyfluthrin technical.

Endpoints	**Beta-Cyfluthrin technical [µg/L]**
LC_{50} (95 % C.L.) (96 h)	3.2 (2.49-4.0)
NOEC (96 h)	0.38

B. OBSERVATIONS

Treatment effects occurred at 0.75 µg/L and higher concentrations and comprised effects on behaviour, pigmentation, respiration and co-ordination. At these exposure levels, the symptoms were exhibited within the initial two hours of exposure but they were not sustained and at 96 hours, all of the surviving fish appeared to have recovered and were normal.

At the highest nominal concentration (6.0 µg/L) employed in the test, 100 % mortality occurred. The effects on mortality of beta-cyfluthrin in the bluegill sunfish are summarised below.

Table B.9.2-5: Effects on mortality of beta-cyfluthrin in the bluegill sunfish

Beta-Cyfluthrin (µg/L, nom.)	**Cumulative mortality (initial population = 7 fish/concentration)**						
	2h	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0
0.75	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0
3.0	0	0	3	3	3	3	43
6.0	0	0	7	7	7	7	100

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) remained within acceptable limits throughout the study.

III. CONCLUSION

The 96-hour LC_{50} for the bluegill sunfish (*Lepomis macrochirus*) exposed to beta-cyfluthrin technical under static conditions is 3.2 µg/L (nominal; 95 % C.L. 2.49-4.0 µg/L). The NOEC (96 h) is 0.38 µg/L beta-cyfluthrin technical. Based on mean measured concentrations the 96-hour LC_{50} is 0.87 µg/L beta-cyfluthrin and the NOEC is 0.103 µg/L.

KIIA 8.2.1/12 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Beta-Cyfluthrin – acute toxicity to common carp
Date:	10 March 2006f
Doc ID:	M-482363-01-1
Report no.:	IRV0120/053830
Guidelines:	EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Deviations: As the measured concentration of the substance decreased, 10 and 19 % of their nominal values at 48 hours and between 5 and 9 % of nominal at 96 hours, endpoints based on nominal values are not appropriate. Therefore, endpoints based on mean measured values were calculated by the RMS. Other validity criteria according to the current OECD Guideline No. 203 are fulfilled.

Dates of experimental work: 21 July 2005 to 12 November 2005

Executive Summary

The acute effects of beta-cyfluthrin to common carp (Cyprinus carpio) were investigated under static conditions. This exposure regime was selected in order to be relevant to a single exposure loading in the field.
Groups of seven juvenile fish were exposed to beta-cyfluthrin technical, dispersed in water at nominal concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. One untreated control and one additional control group containing dimethyl-formamide and water (100 µL/L) were included in the study. Observations of the fish were made after approximately 2, 4, 24, 48, 72 and 96 hours of exposure.
At the start of the test, the measured concentrations ranged between 95 and 129 % of their nominal values; thereafter, the measured concentrations decreased, ranging between 10 and 19 % of their nominal values at 48 hours and between 5 and 9 % of nominal at 96 hours. The average recovery rate was 20.84 %.

Based on nominal concentrations, the 96-hour LC_{50} is > 6.0 µg/L beta-cyfluthrin and the NOEC is 0.38 µg/L. Based on mean measured concentrations the 96-hour LC_{50} is 1.25 µg/L beta-cyfluthrin and the NOEC is 0.079 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Description:	Powder
Lot/Batch #:	030916
Purity:	96.1 %

2. Vehicle and/or positive control: None

3. Test organism:

Species:	Common carp (Cyprinus carpio)
Age:	Juvenile
Size:	mean fork length: 4.0 cm
Body weight of the animals:	mean wet weight: 2.0 g
Loading:	initial static loading of 0.64 g body weight/L
Source:	Commercial fish farm in the UK
Culture:	Stock of fish obtained from supplier on 15 April 2005 and held in an aerated supply of diluent water under flow-through conditions until use.
Diet/Food:	Standard commercial fish food (Nishikoi pellets) in an amount equivalent to 1.5 % of the total wet weight of fish in the hold ing tank and supplemented with bloodworms. Food was sup plied daily until 24 hours prior to testing.
Acclimation period:	14 days

4. Environmental conditions:

Temperature:	Control (min-max): 21.5-23.2 solvent control (min-max): 21.7-23.1 0.38 µg/L (min-max): 21.8-23.2 0.75 µg/L (min-max): 21.8-23.1 1.5 µg/L (min-max): 21.8-23.0 3.0 µg/L(min-max): 21.8-23.1 6.0 µg/L (min-max): 21.7-23.0
Photoperiod:	16 hours light, 8 hours dark
pH:	Control (min-max): 7.98-8.22 solvent control (min-max): 7.99-8.22 0.38 µg/L (min-max): 8.01-8.35 0.75 µg/L (min-max): 8.05-8.39 1.5 µg/L (min-max): 8.06-8.46 3.0 µg/L(min-max): 8.11-8.39 6.0 µg/L (min-max): 8.12-8.32
Dissolved oxygen:[% air saturation value]	Control (min-max): 93-104 solvent control (min-max): 84-103 0.38 µg/L (min-max): 92-103 0.75 µg/L (min-max): 94-104 1.5 µg/L (min-max): 94-103 3.0 µg/L(min-max): 89-103 6.0 µg/L (min-max): 87-102
Conductivity:	350 µS/cm³ in the dilution water at 20 °C
Total hardness:	190-192 mg/L as CaCO3

B. STUDY DESIGN AND METHODS

1. Experimental treatments

A range finding test was followed by a definitive test with five test concentrations. Control groups were exposed to diluent water alone and diluent water plus auxiliary solvent (100 µL dimethylformamide/L). The definitive test was conducted at nominal beta-cyfluthrin technical concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L. In the definitive test, groups of seven fish were placed at random into each glass aquarium containing the prepared control or test media (fish were added at the same time as the test substance). Each vessel contained 22 litres of medium to a depth of 22 cm. This provided an initial static loading of 0.64 g bodyweight/L. The fish were exposed to the control or test conditions for a period of 96 hours under static conditions, with no renewal of media.

Treatment and control groups were maintained at 20 ± 2 °C throughout the exposure period and constant to within ± 1 °C during the study. Supplementary aeration was provided via narrow bore glass tubes. A photoperiod of 16 hours light: 8 hours dark was maintained, with periods of subdued lighting at the beginning and end of each light phase. The fish were not fed during the 96 hour exposure period.

2. Observations
The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle. In addition to observations on mortality at approximately 2, 4, 24, 48, 72 and 96 hours, subjective assessments were also made on the incidence and type of any sub-lethal effects compared with control fish. Daily records of temperature, pH and dissolved oxygen were kept performed for each control and test vessel. Total hardness was measured at 0 hours in the control and the highest test concentration.

3. Statistical calculations
The LC_{50} was calculated using the SAFEStat LD_{50} application (SAS 8.2.) and the nominal concentrations. The NOEC was derived by direct inspection of the data for lethal and treatment-related effects. An incidence rate of more than one affected fish out of seven is considered to be significant.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The measured concentrations of beta-cyfluthrin ranged between 95 and 129 % of their nominal values in samples of freshly prepared media. Thereafter, the measured concentrations decreased, ranging between 10 and 19 % of their nominal values at 48 hours and between 5 and 9 % of nominal at 96 hours.

The 96 hour LC_{50} and NOEC values are presented below. The test results are expressed in terms of the nominal concentrations of beta-cyfluthrin technical.

Endpoints	Beta-cyfluthrin technical [µg/L]
LC_{50} (95 % C.L.) (96 h)	> 6.0 (-)
NOEC (96 h)	0.38

B. OBSERVATIONS
All validity criteria according to OECD 203 were fulfilled, as mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

Treatment effects were observed at 0.75 µg/L and higher concentrations and comprised effects on behaviour, pigmentation, respiration and co-ordination. At these levels, the symptoms were exhibited within the initial two hours of exposure but they were not sustained and at 96 hours, all of the surviving fish appeared to have recovered and were normal.

At the highest nominal concentration (6.0 µg/L) employed in the test, 14 % mortality occurred. The effects on mortality of beta-cyfluthrin in the common carp are summarised below.

Table B.9.2-6: Effects on mortality of beta-cyfluthrin in the common carp

Beta-Cyfluthrin (µg/L, nom.)	Cumulative mortality (initial population = 7 fish/concentration)						
	2h	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0
0.38	0	0	0	0	0	0	0

0.75	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0
3.0	0	0	0	0	0	0	0
6.0	0	0	0	1	1	1	14

The measurements of water quality (temperature, pH, concentrations of dissolved oxygen and total hardness) remained within acceptable limits throughout the study.

III. CONCLUSION

The 96-hour LC_{50} for the common carp (*Cyprinus carpio*) exposed to beta-cyfluthrin technical understatic conditions is > 6.0 µg/L (nominal). The NOEC after 96 h is 0.38 µg technical/L beta-cyfluthrin. Based on mean measured concentrations the 96-hour LC_{50} is 1.25 µg/L beta-cyfluthrin and the NOEC is 0.079 µg/L.

Metabolites of beta-cyfluthrin

KIIA 8.2.1/05

Author:	[redacted]
Title:	Acute toxicity of Dichlorovinylcarboxylic acid to rainbow trout
Date:	7 September 1984
Doc ID:	M-034724-01-1
Report no.:	515
Guidelines:	US-EPA FIFRA § 72-1 guideline
GLP:	yes
Validity:	Valid

Deviations: The study is valid according to the current OECD Guideline No. 203. No analytic on the test substance was conducted. However, DCVA is stable in aquatic environment (see Volume_3CA_B-8.2.2).
Test material: DCVA (Dichlorovinylcarboxylic acid), purity: 99.9 %, batch no. 150-6-52
Results: The 96-hour LC_{50} and the lowest lethal concentration in rainbow trout were greater than 14700 µg/L (nominal). The no observed effect concentration was 14700 µg/L.

Conclusion: LC_{50} (96 h) > 14700 µg/L; NOEC (96 h) = 14700 µg/L

KIIA 8.2.1/06

Author:	[redacted]
Title:	Acute toxicity of Fluorphenoxybenzaldehyde to rainbow trout
Date:	3 August 1984
Doc ID:	M-034806-01-1
Report no.:	502
Guidelines:	US-EPA FIFRA § 72-1 guideline
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 203. No analytics on the test substance were conducted. Still, the study can be used to address the acute toxicity of the metabolite to fish (see Volume1 section 2.9.2.1)

Test material: FPB-aldehyde (Fluorphenoxybenzaldehyde), purity: 96.2 %, batch no. 150-6-51

Results: The 96-hour LC_{50} and the lowest lethal concentration were 792 µg/L and 655 µg/L, respectively. The no observed effect concentration was 410 µg/L.
Conclusion: **LC_{50} (96 h) = 792 µg/L; NOEC (96 h) = 410 µg/L**

KIIA 8.2.1/14

Author:	Hill, I. R.
Title:	Aquatic organisms and pyrethroids
Date:	1990
Doc ID:	M-090574-01-1
Report no.:	Lit. 6002
Guidelines:	Publication: Society of Chemical Industry, Great Britain, Journal: Pesticide Science, Volume:27, Pages:429-457, Year:1990
GLP:	no
Validity:	supplemental

Results: The FPB-acid 96 h LC50 for rainbow trout is reported to be 13000 µg/L.
Conclusion: **supplemental information**

KIIA 8.2.1/13 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	Acute toxicity of beta-Cyfluthrin FPB-acid (tech.) to fish (Oncorhynchus mykiss) under static conditions
Date:	23 February 2010
Doc ID:	M-364414-01-1
Report no.:	EBFRL003
Guidelines:	US-EPA FIFRA § 72-1 guideline, SEP-EPA-540/9-85-006 (1982/1985), OPPTS 850.1075 (Public Draft, 1996), EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
GLP:	yes
Validity:	valid

Guideline: US-EPA FIFRA § 72-1 guideline, SEP-EPA-540/9-85-006 (1982/1985), OPPTS 850.1075 (Public Draft, 1996), EU Directive 92/69/EEC, C.1 (1992), OECD Guideline No. 203 (rev.1992)
Deviations: None

Executive Summary
The effect of FPB-acid to rainbow trout (*Oncorhynchus mykiss*) was evaluated in a 96-hour static toxicity test conducted at nominal (mean measured) concentrations of 1.00 (1.14), 2.00 (1.75), 4.00 (4.36), 8.00 (6.79), 16.0 (11.9) and 32.0 (28.9) mg/L. Ten fish were exposed in the control and in each treatment. Samples were analysed for FPB-acid at 0 hours (before fish addition) and after 48 and 96 hours. Initial recovery was between 62 % and 150 % of nominal and between 91 % and 97 % at the end of the test after 96 hours. All results are based on mean measured concentrations as some of the analysed results revealed recoveries out of the range of 80 – 120 % of nominal.
In the controls no mortalities or sub-lethal findings were observed. In all test levels ≥ 1.14 mg /L FPB-acid behavioural changes were observed during the entire exposure period
All validity criteria according to the guideline OECD 203 were fulfilled.

The 96-hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed FPB-acid was calculated to be 4.06 mg/L (mean measured) with 95 % confidence interval of 1.69 to 8.36 mg/L. The NOEC (96 h) was < 1.14 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	beta-Cyfluthrin FPB-acid
Description:	White powder
Lot/Batch #:	SES 10570-2-1
Purity:	99.2 %

2. Vehicle and/or positive control: Filtered and dechlorinated tap water

3. Test organisms:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Age:	Juvenile
Size:	mean: 5.1 cm ± 0.5 cm
Weight:	mean: 1.5 g/fish ± 0.6 g
Source:	[redacted]
Loading:	0.38 g fish/L (10 fish per 40 litres of test medium)
Diet/Food:	no feeding for 48 hours prior to test and during the total test period
Acclimatisation:	14 days + 48 hours before testing

4. Environmental conditions:

Temperature:	10.7 – 11.3 °C
Photoperiod:	16 hours
pH:	
Control (start – 96 h):	7.3 - 7.1
1.14 mg/L(start – 96 h):	7.3 – 6.9
1.75 mg/L (start – 96 h):	7.1 – 6.9
4.36 mg/L (start – 96 h):	6.9 – 7.0
6.79 mg/L(start – 96 h):	6.9 – 7.1
11.9 mg/L (start – 24 h):	6.8
28.9 mg/L (start):	6.5
Dissolved oxygen:	83 – 99 % O_2
Conductivity:	< 0.2 mS/cm³ in the dilution water
Hardness:	40 - 60 mg CaCO3/L

B. STUDY DESIGN

1. Experimental treatments

The toxicity test was performed at nominal (mean measured) concentrations of 1.00 (1.14), 2.00 (1.75), 4.00 (4.36), 8.00 (6.79), 16.0 (11.9) and 32.0 (28.9) mg test item/L. The test was conducted under static test conditions. A negative control (dilution water only) was also prepared. A single replicate vessel was prepared for the control and at each treatment level, each containing ten fish (added to 40 L test medium).

2. Observations

Fish in all vessels were observed for sublethal effects and mortality after 4, 24, 48, 72 and 96 hours. Temperature, pH-value and oxygen saturation of test solutions were measured on a daily basis. Hardness and conductivity of the test water was measured at test initiation. Analytical measurements were performed by HPLC analysis at test initiation and after 48 and 96 hours.

3. Statistical calculations
The LC_{50} values and their 95 % confidence intervals were calculated using one of three statistical techniques: moving average, logit analysis or probit analysis. The NOEC was determined by visual interpretation of the mortality and observation data.

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical data: The analytical determination of FPB-acid (in water by HPLC - UV) revealed initial recovery values between 62 % and 150 % of nominal and between 91 % and 97 % at the end of the test after 96 hours. All results are based on mean measured concentrations as some of the analysed results revealed recoveries out of the range of 80 – 120 % of nominal.

Table B.9.2-7: Nominal and measured concentrations of FPB-acid

Nominal conc. test item [mg / L]	Nominal concentration of FPB-acid [mg / L]	Measured concentration of FPB-acid					
		day 0 [mg / L]	day 2 aged [mg / L]	day 2 new [mg / L]	day 4 [mg / L]	Mean day 0 – day 4 [mg / L]	Percent of nominal [%]
1.00	0.992	1.49	1.24	0.934	0.900	**1.14**	115
2.00	1.98	1.48	1.79	1.89	1.85	**1.75**	88
4.00	3.97	3.18	6.57	3.90	3.77	**4.36**	110
8.00	7.94	4.97	6.85	7.63	7.70	**6.79**	86
16.00	18.9	9.84	11.5	14.4	-	**11.9**	75
32.00	31.7	21.5	36.3	-	-	**28.9**	91

The 96 hour LC_{50}, NOEC and NOLEC values are presented below.

Endpoints	Beta-cyfluthrin technical [µg/L]
LC_{50} (95 % C.L.) (96 h)	> 6.0 (-)
NOEC (96 h)	0.38

B. OBSERVATIONS

No mortalities or sub-lethal findings were observed in the controls. In all test levels ≥ 1.14 mg /L FPBacid (mean measured) behavioural changes were observed during the entire exposure period. After 96 hours of exposure fish showed the following behavioural symptoms:

- showed laboured respiration
- remaining for unusually long periods on the bottom of the aquarium
- showed loss of equilibrium with lateral deviation from their normal orientation
- turned in a vertical position

All measured water quality parameters were within the specifications recommended by the OECD 203 test guideline. Dissolved oxygen concentrations ranged from 83 to 99 % oxygen saturation, the pH values ranged from 6.5 to 7.3 and the water temperature ranged within 10.7 – 11.3 °C in all aquaria over the whole test period.

The results for mortality are presented in the table below.

Table B.9.2-8: Effects of beta-cyfluthrin FPB-acid to rainbow trout

FPB-acid [mg/L, mean measured]	Cumulative mortality (initial population = 710fish/concentration)					
	4h	24 h	48 h	72 h	96 h	%
Control	0	0	0	0	0	0
1.14	0	0	0	0	0	0
1.75	0	0	0	2	2	20
4.36	0	0	0	1	1	10
6.79	0	0	4	7	7	70
11.9	4	10	10	10	10	100
28.9	10	10	10	10	10	100

All validity criteria according to OECD 203 were fulfilled, as mortality within the 48-hour setting-in period did not exceed 5 %, mortality in control group did not exceed 10 % (or one fish if less than ten are used), dissolved oxygen concentration was ≥ 60 % of air saturation and constant exposure conditions have been maintained.

III. CONCLUSIONS

The 96 hour LC_{50} for rainbow trout (*Oncorhynchus mykiss*) exposed FPB-acid was calculated to be 4.06 mg/L (mean measured) with 95 % confidence interval of 1.69 to 8.36 mg/L. The NOEC (96 h) was < 1.14 mg/L.

B.9.2.2 Long-term and chronic toxicity to fish

For the chronic effects to fish, reference is made to studies with cyfluthrin on which the current Annex I listing of beta-cyfluthrin is based.
Adjusting the endpoints to beta-cyfluthrin, they have to be multiplied with 0.42 due to the lower content of the active (fish toxic) diasteromer pairs II and IV in cyfluthrin (see also the introducing part).
Although there is evidence of racemisation in water and thus, a partial conversion from diastereomer pair II to I and IV to III from the mesocosm studies of Heimbach 1989 and 1990, this does not generally justify the assumption that beta-cyfluthrin converts into the less toxic cyfluthrin when sprayed into water bodies. Likewise, raw data from the hydrolysis study by Krohn 1997a (see Volume_3CA-B-8.2.1) do not exclude a slight epimerisation process in general, but do not provide clear evidence for epimerisation. Due to the study design it is not possible to distinguish between the processes of hydrolysis and epimerisation.
The reaction pathway of the racemisation of the closely related pyrethroid cypermethrin is well described in Nillos et al. 2009[5]. According to this publication, the reaction is due to a positive partial charge at the α-C atom. Therefore, this carbon is relatively acidic leading to an easy loss of the C-bound proton and consequently creating an anion. The reprotonation can occur from both sides. However, Nillos et al. (2009) used alcoholic (protic) solvents being responsible for reprotonation and stated that the epimerisation was not observed in pure water. Moreover, the authors cited Perschke and Hussain (1992) reporting that the addition of HCl prevented the isomerisation in case of deltamethrin. Overall, it is not clear which further components are needed to support this racemisation. Further more it appears quitelikely that an alkaline pH favours the racemisation since the separation of the proton from the α-C atom is supported by more alkaline conditions.
The mentioned mesocosm studies Heimbach 1989 and 1990 showed slight to moderate alkaline conditions. Therefore, no conclusion can be drawn for the fate of isomers in neutral or slightly acidic water bodies.
Concerning the ELS-study by [redacted] (1985) the effects observed argue against waiving the adjustment of cyfluthrin to beta-cyfluthrin. Considering the type of effects [significantly reduced number of swim ups (only 10 %) followed by death rate of 100 % (0.4 µg/L) and still high mortalities for the next lower applied concentrations] it cannot be excluded that the toxic effects are mainly attributed to

[5] Nillos et al. 2009

the exposure of fish embryos (fish eggs) within the hatching stage. As the duration of this sensitive stage is quite short, fish embryos can be exposed to beta-cyfluthrin even if conditions within some water bodies are favourable to epimerisation.

Hence, the adjustment of the enpoint from cyfluthrin to beta-cyfluthrin is regarded necessary to maintain a sufficient protection level.

Table B.9.2-9: Chronic toxicity of beta-cyfluthrin and cyfluthrin to fish

Species	Test design	LC50 (µg as/L)	NOEC (µg as/L)	Reference	reliability
Cyfluthrin					
Oncorhynchus mykiss	**58 d ELS flow-through**	**0.069 (mm)**	**0.010 (mm)** **adjustment to beta-cyfluthrin: 0.0042 (mm)**	**KIIA 8.2.4 683 [redacted], 1985 M-008695-01-1 R-19088**	**valid**
Pimephales promelas	307 d FLC flow-through	-	0.140 (mm) adjustment to beta-cyfluthrin: 0.0588 (mm)	KIIA 8.2.5 100097 [redacted], 1990 M-022913-02-1 R-34700	valid

Studies shaded in grey have been reviewed as part of the 2002 EU evaluation.
Values in **bold**: Endpoints used for risk assessment
mm: mean measured

Early life stage toxicity test

Annex I inclusion of beta-cyfluthrin is based on an early life stage study with rainbow trout (Carlisle, 1985) conducted with cyfluthrin.

KIIA 8.2.4

Author:	[redacted]
Title:	Toxicity of Cyfluthrin (Baythroid) technical to early life stages of rainbow trout
Date:	1985
Doc ID:	M-008695-01-1
Report no.:	683
Guidelines:	Equivalent to the test guidelines US-EPA FIFRA § 72-4 guideline and OECD Guideline No. 210
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD 210 guideline, with some minor deviations not influencing the outcome of the study. The dissolved oxygen concentration stayed within the targeted limits of 6.5 to 11.9 ppm (three occasions above that range). The temperature during the study ranged from 8.3 to 11.9 °C (recommended 8.5 to 11.5).
Test material: Cyfluthrin techn. (Baythroid), purity: 96.0 %, batch no. 84-R-221-1/7

Results: The early life stages of rainbow trout were exposed to five concentrations of cyfluthrin for 58

days, i.e. nominal 0.025, 0.050, 0.100, 0.200 and 0.400 µg/L.
The mean measured concentration ranged from 32 % to 48 % of nominal.
During the period of exposure, there were no concentration-related embryonic deaths, but larvae mortality at the three highest concentrations tested. Growth as measured by biomass and mean fish weight was significantly reduced in the 0.050, 0.100, 0.200 µg/L groups (0.0177, 0.0318 and 0.0848 µg/L based on mean measured concentrations). The number of fish showing behavioural signs at concentrations of nominal 0.050 µg/L and higher was statistically significantly increased.
The no observed effect concentration was 0.010 µg/L (based on mean measured concentration) or 0.025 µg/L (nominal).
Due to the significantly reduced number of swim ups (only 10 %) followed by death rate of 100 % (0.4 µg/L nominal) and still high mortalities for the next lower applied concentrations, it can be assumed that the toxic effects are mainly attributed to the exposure of fish embryos (fish eggs) within the hatching stage.

Conclusion:
The 56 day LC_{50} of cyfluthrin is 0.069 µg/L (mm).
The NOEC of cyfluthrin is 0.01 µg/L.
Endpoints for beta-cyfluthrin are adjusted by multiplication with the factor 0.42:
LC_{50} (56 d) = 0.029 µg/L

NOEC (56 d) = 0.0042 µg/L

CA 8.2.2.2 Fish full life cycle test

Annex I inclusion of beta-cyfluthrin is based on a full life-cycle study with fathead minnow (Rhodes *et. al*, 1990) conducted with cyfluthrin.

KIIA 8.2.5

Author:	[redacted]
Title:	Full Life-Cycle Toxicity of 14C-Cyfluthrin (Baythroid®) to the Fathead Minnow (Pimephales promelas) Under Flow-Through Conditions
Date:	2 April 1990
Doc ID:	M-022913-02-1
Report no.:	100097
Guidelines:	US-EPA FIFRA § 72-4 guideline, 40 CFR, Section 158.145
GLP:	yes
Validity:	valid

Guideline: US-EPA FIFRA § 72-4 guideline, 40 CFR, Section 158.145

Deviations: The study is valid according to the current US EPA protocol OPPTS 850.1500 Fish life cycle toxicity. A mortality rate of 37.5 % in the control group 153 -301 days post-hatch was determined. Hence, data about survival 153 -310 d are considered as not fully reliable.

Test material: ^{14}C-Cyfluthrin (Baythroid), purity: 99.0 %, reference no. PS-2344

Results: The study was initiated using newly fertilised eggs (<24 hours post-fertilisation) with exposure continuing for 301 days post-hatch. Mean measured exposure concentrations, determined by liquid scintillation counting techniques (LSC), were 0.018, 0.033, 0.065, 0.14 and 0.29 µg as/L. These mean values ranged from 106 to 116 % of the nominal concentrations of 0.016, 0.031, 0.063, 0.13 and 0.25 µg as/L. Of the 82 % average 14C-activity recovered, 90 % was characterised as ^{14}C-cyfluthrin. No significant difference in parental generation hatchability was exhibited, while F1 egg hatchability

was significantly reduced at the highest concentration tested. Survival, in both parental and F1 generations, was significantly reduced at approximately 60-days post-hatch at the highest concentration tested. Survival was not significantly reduced at any other interval. Growth, as reflected by standard length and wet weight was not significantly reduced compared to the control in either the parental or F1 generation at any concentration tested. Reproductive success, as measured by the number of spawns, number of eggs, number of eggs/spawn, number of reproductive days and the number of eggs/pair/reproductive day, was not significantly reduced at any of the four concentrations examined. The no observed effect concentration (NOEC) was 0.14 µg as/L (based on mean measured concentration or nominal 0.13 µg as/L).

Conclusion: The NOEC (mm) of cyfluthrin is 0.14 µg/L.
The Endpoint for beta-cyfluthrin adjusted by a multiplication factor of 0.42 is as follows:

NOEC (mm) = 0.059 µg/L

B.9.2.2.1 Bioconcentration in fish

The log P_{ow} values of the isomers contained in beta-cyfluthrin are greater than 3, i.e. 5.9 for isomer II and 5.8 for isomer IV (at 25 °C). Therefore a bioconcentration study in fish is required. Annex I inclusion of beta-cyfluthrin is based on a 28-day bioconcentration study with the bluegill sunfish conducted with cyfluthrin (Carlisle and Roney, 1984, KIIA 8.2.6.1/01).
Due to deficiencies in the existing study, a new bioconcentration study in fish was submitted and is summarised below.

Species	Test design	Result			Reference	Reliability
Beta-Cyfluthrin						
Lepomis macrochirus	28-day flowthrough	BCFSS	steady-state [L/kg]	1458	CA 8.2.2.3/02 D78913 [redacted], 2014 M-481021-01-1 R-33370	valid
		BCFSSL	lipid-normalised steady-state[L/kg]	1695		
		BCFSSL (parent)	lipid-normalised steady-state[L/kg] based on parent substance	2295		
		BCFkL	lipid-normalised kinetic [L/kg]	1745		
		BCFkLg	lipid-normalised growth-corrected kinetic [L/kg]	**1822**		

Metabolites of beta-cyfluthrin
For the metabolites FPB-acid and DCVA new studies on partition co-efficient (n-octanol/water) are available. For FPB-acid the maximum log P_{ow} was 2.6 at pH 5 (23 °C) and for DCVA the maximum log P_{ow} was 2.5 at pH 5 (25 °C).
Accordingly, no studies on the bioconcentration of beta-cyfluthrin metabolites are needed for Annex I renewal.

KIIA 8.2.6.1/02 (newly submitted with renewal dossier)

Author:	[redacted]
Title:	[Fluorophenyl-14C]Beta-Cyfluthrin: Bioconcentration Test in the Bluegill Sunfish (Lepomis Macrochirus) under Flow-Through Conditions
Date:	3 March 2014
Doc ID:	M-481021-01-1
Report no.:	D78913
Guidelines:	OECD Guideline No. 305
GLP:	yes
Validity:	valid

Executive Summary

The bioconcentration and depuration characteristics of beta-cyfluthrin were investigated in the Bluegill sunfish in a dynamic flow-through system. The bioconcentration in whole fish was calculated. The fish were continuously exposed to [Fluorophenyl-^{14}C]beta-cyfluthrin at an average concentration of 0.12 µg eq/L for 28 days. After the exposure, the fish were transferred to flowing untreated water and the depuration of radioactivity was followed for further 28 days.
Temperature, pH and oxygen concentrations were monitored from day 0 to day 56 and were within acceptable limits; measurements ranged from 22.0 - 22.7 °C, 8.0 - 8.4 and 6.1 - 8.4 mg/L, respectively. The radioactive residues during exposure in whole fish increased rapidly (0.120 µg eq/g on day 14 and 0.121 µg eq/g on day 20) and ranged between 0.158 µg eq/g and 0.186 µg eq/g for time intervals 24 to 28 days (plateau phase). However, depuration was delayed with a depuration half-life of 8.66 days (0.052 µg eq/g on depuration day 4 and 0.017 µg eq/g on depuration day 28).

BCFss and kinetic BCFk were calculated to be 1458 and 1508, respectively. The lipid normalised BCFL based on BCFk was 1753. The growth corrected BCF values BCFkg and BCFkLg were 1640 and 1907, respectively. All these data were based on total radioactive residues.
BCF_{SS} based on the real measured fraction of the parent compound is 1974. The corresponding lipid normalised BCF_{SSL} is 2295.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	[fluorophenyl-UL-14C]Beta-cyfluthrin
Lot/Batch #:	KML 9609
Specific activity:	4.36 MBq/mg
Radiochemical purity:	> 98 % (sum of isomers)
2. Vehicle and/or positive control:	Acetone
3. Test organism:	
Species:	Bluegill sunfish (*Lepomis macrochirus*)
Age:	Adult
Size: mean length:	3.75 cm
Body weight of the animals:	mean weight: 0.804 g
Loading:	initial loading of 0.32 g body weight/L (Based on a daily flow through volume of 250 L)
Source:	[redacted]
Diet/Food:	During the test, the fish was fed once daily (TetraMin, Tetra GmbH, D49304 Melle, containing 8.0 % lipid and 48.0 % total protein), based on about 2 % of the average fish body weight, taking into account increasing body weights and decreasing number of fish per tank.
Acclimation period:	5 weeks in tap water

4. Environmental conditions:

Temperature:	
Control (min-max):	22.2-22.5
Treatment (min-max):	22.0-22.7
Photoperiod:	16 hours light, 8 hours dark, light intensity at light period approximately 300-400 Lux
pH:	
Control (min-max):	8.1-8.4
Treatment (min-max):	8.0-8.4
Dissolved oxygen [mg/L]:	

Control (min-max):	6.6-8.4
Treatment (min-max):	6.1-8.3
Total hardness:	
Control:	9.5°d
Treatment:	9.0°d

B. STUDY DESIGN

1. Experimental conditions

The fish were continuously exposed to [Fluorophenyl-14C] Beta-cyfluthrin at an average concentration of 0.12 µg eq/L for 28 days (in µg parent equivalents/L). Due to the low water solubility of the test item and the toxicity to fish, no higher concentration could be tested. After the exposure, the fish were transferred to flowing untreated water and the depuration of radioactivity was followed for further 28 days. Temperature, pH and oxygen concentrations were monitored from day 0 to day 56 and were within acceptable limits; measurements ranged from 22.0 - 22.7 °C, 8.0 - 8.4 and 6.1 - 8.4 mg/L, respectively.
The minimal duration of the uptake phase can be calculated according to the OECD Guideline 305. Based on the log POW, the expected depuration rate constant (k_2) and the optimal duration of the uptake phase (u = 95 % of steady state) are defined as:
log k2 = -0.414 log (P_{OW}) + 1.47 and u = 3.0/k2
Based on a log P_{OW} value of 5.9, a theoretical k_2 of 0.107 can be calculated. Based on k_2 approximately 28 days would be needed to reach 95 % of "steady state". Therefore, an accumulation period of 28 days was selected which seems to be sufficient to reach steady state (= plateau level).
To reach 95 % depuration, theoretically a depuration period of 28 days would be needed (ln 0.01/-k_2). Therefore, a depuration period of 28 days was selected.

2. Observations

Water samples were taken from the central area of the respective test tank before feeding and immediately before fish sampling. Additionally, at selected time intervals water samples were also drawn from the corresponding mixture chamber.
During the accumulation phase, fish were sampled on Day 4, 8, 14, 20, 24 and 28. During the depuration phase, fish were sampled on day 32, 40, 48 and 56.
On each sampling occasion, six fish were collected randomly from each exposure tank, rinsed with water, sacrificed in 1.5 % (v/v) 2-phenoxy-ethanol in purified water and blotted dry and weighed. At two time intervals (after 20 and 28 days of uptake) 8 fish were sampled and stored at approximately -20 °C for additional analyses.

3. Calculations

Details on the calculations on depuration kinetics and bioconcentration kinetics are given in the report.

II. RESULTS AND DISCUSSION

A. FINDINGS

All validity criteria according to OECD 305 were fulfilled by meeting the following criteria:
Temperature variation was less than ± 2 °C (22.0-22.7 °C).
The concentration of dissolved oxygen should not fall below 60 % saturation, i.e. not below 5.03 - 5.50 mg O_2/L at 20 - 25 °C (>6.1 mg/L).
The variation of the tank concentration during exposure was slightly higher than ± 20 %. However, on one day the measured concentration was above this range. During the accumulation period, total radioactivity levels remained sufficiently constant to show equilibrium. Total radioactivity level amounted on average to 0.12 ± 0.02 µg eq/L over the 28 days (Table 6). One measurement was too high due to a malfunction in the flow system (day 22). The variation was slightly higher than ± 20 %.
In consequence, instead of 0.12 µg/L, a level of 0.18 µg/L beta-cyfluthrin was determined in the fish tank at day 22.
In addition this peak near the end of the uptake phase masks the plateau concentration in fish. It should

be noted that the test report does not consider this increase in test substance concentration as a significant deviation to study plan. The original evaluation is based on the mean concentration of the last three sampling dates of the uptake phase (0.155 µg/L, based on days 20, 24 and 28).

Additionally, the following parameters were within the required limits:
The pH values were within an acceptable range in the two tanks (8.0-8.4)
The particle content of the tap water (dry matter not passing a 0.45 µm filter) was on average 1.6 mg/L (n = 2).

The TOC value of the untreated water was on average <0.1 mg/L. A TOC value of approx. 49 mg/L can be expected from the used solvent (acetone, 0.01 %). During the test (uptake day 0 to 28), the TOC value did not exceed the concentration of organic carbon originating from the test item and the solubilising agent by more than 10 mg/L (± 20 %). The values were even lower than the expected value probably due to evaporation from the tanks.
The radioactive residues during exposure in whole fish increased rapidly (0.120 µg eq/g on day 14) and ranged between 0.158 µg eq/g and 0.186 µg eq/g for time intervals 24 to 28 days). However, depuration was first quick (0.052 µg eq/g on depuration day 4) and later delayed (0.017 µg eq/g on depuration day 28). The results based on total radioactive residue (TRR) are summarised as follows:

Parameter	Description	Value
kg	growth rate constant [day-1], based on depuration data	0.007
k1	overall uptake rate constant [L kg-1 day-1]	131.2
k2	overall depuration rate constant [day-1], based on depuration data	0.087
k2g	growth-corrected depuration rate constant [day-1]	0.08
CfSS	chemical concentration in fish at steady state [µg L-1]	0.172
Cw	chemical concentration in the water [mg L-1]	0.12
Ln	lipid normalisation factor	1.163
BCFSS	steady-state BCF [L kg-1]	1458
BCFSSL	lipid-normalised steady-state BCF [L kg-1]	1695
BCFSS (parent)	staedy-state BCF [L kg-1] based on measured concentration of the parent substance*	1974
BCFSSL (parent)	lipid-normalised staedy-state BCF [L kg-1] based on measured concentration of the parent substance*	2295
BCFkL	lipid-normalised kinetic BCF [L kg-1]	1754
BCFkLg	lipid-normalised growth-corrected kinetic BCF [L kg-1]	**1822**
t0.5g	growth-corrected half-life [day]	8.66

* In the exposure water, the test item was measured in amounts ranging from 50.7 to 64.9 % of the total radioactivity.

Exposure concentration

During the accumulation period, total radioactivity levels remained sufficiently constant to show equilibrium. Total radioactivity level amounted on average to 0.12 ± 0.02 µg eq/L over the 28 days. One measurement was too high due to a malfunction in the flow system (day 22). The variation was slightly higher than ± 20 %. However, due to the need of performing a study at the very low level of 0.12 µg/L (due to low solubility and high toxicity), a slightly higher variation than required can be accepted.
In consequence, instead of 0.12 µg/L a level of 0.18 µg/L beta-cyfluthrin was determined in the fish tank at day 22.
In addition, this peak near the end of the uptake phase masks the plateau concentration in fish. It should be noted that the test report does not consider this increase in test substance concentration as a significant deviation to study plan. The original evaluation is based on the mean concentration of the last three sampling dates of the uptake phase (0.155 µg/L, based on days 20, 24 and 28). Since the RMS does not agree with this conclusion, a $BCF_{steady\ state}$ based on the mean concentration between days 24 and 28 was calculated.

Very small amounts of radioactivity were measured in the tank water during depuration. Values

ranged from 0.011 µg eq/L (day 4 of depuration) to <LOQ. The measured radioactivity on day 4 of depuration reflects the on-going depuration of the radioactivity from the fish.
Radioactivity in the control tank was <LOQ at all time intervals.

Residue in fish
The radioactive residues during exposure in whole fish increased rapidly (0.120 µg/g on day 14 and 20) and ranged between 0.158 µg eq/g and 0.186 µg eq/g for time intervals 24 to 28 days. However, depuration was first quick as only 0.052 µg eq/g were measured on depuration day 4 and later delayed 0.017 µg eq/g on depuration day 28.
Taking into account the specific radioactivity of the application solution (4.36 MBq/mg), all control values for fish were < LOQ.

B. OBSERVATIONS

Two fish out of 86 fish died in the treated tank. No mortality was observed in the control tank and no symptoms were observed throughout the study.
Growth rates for the control tank and the test tank were similar with 0.012 and 0.015, respectively. The growth rate of the exposed fish differed significantly between exposure phase and depurations phase. Therefore, for the growth correction the growth rate of 0.007 of the depuration phase was used as recommended by OECD 305.

Based on the depuration data, a depuration half-life of 1.99 days was calculated. Additionally the depuration half-life, based on the k2a obtained from the uptake phase was calculated. This value was calculated to be 7.97 days. The growth corrected half-life was 8.66 days.

Mean lipid concentrations (determined in nine fish) were 41 mg/g wet weight (day 4), 45 mg/g (day 28) and 58 mg/g (day 56).
Based on the radioactivity levels in fish after exposure to [Fluorophenyl-14C]Beta-cyfluthrin at an average dose level of 0.12 µg eq/L, the bioconcentration factor at plateau level (BCFss) amounted to 1458 when considering only the parent concentration.

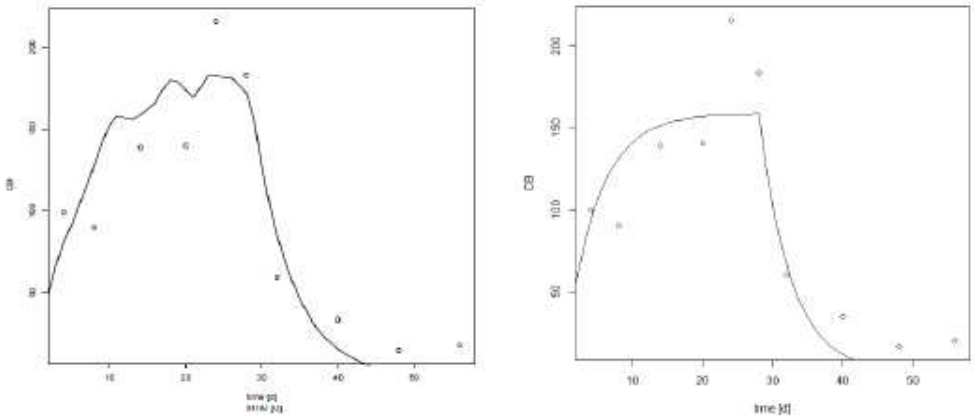
Data from fish indicate that the beta-cyfluthrin uptake meets the first-order kinetics only to a certain degree. However, the analytical results in fish seem to confirm that a steady state would have been approached if the dosing of the test substance had changed during that period.
The calculation of the $BCF_{steady\ state}$ directly relies on the concentration of the test substance at steady state in fish and water, and we agree that this concentration cannot be determined within the new study without a high level of uncertainty. We therefore propose to consider the $BCF_{steady\ state}$ only as additional information, but not directly for the assessment of the substance. The kinetic BCF_k does not depend on the concentrations at only one certain point of time and therefore should provide a more reliable result than the $BCF_{steady\ state}$
It should be noted that an update for the OECD TG 305 is in development, mainly driven by NL, UK and DE. The new document will include guidance for the interpretation of both the steady state and the kinetic BCF values and decision-making support for choosing the relevant BCF calculation method. Currently the drafting of the guidance supports to choose a kinetic BCF instead of a steady state for cases where the steady state concentations or the approach of the steady state shows a high level of uncertainty. Therefore the new guidance will provide valuable information for the assessment of this study and it should be checked later on if this study evaluation is in accordance with the final guidance document.
Original calculation of BCF_k cannot be considered as appropriate; therefore a recalculation was performed both by the RMS.
According to OECD TG 305 there exist two possibilities to calculate k1 and k2, either simultaneously or sequentially (starting with k2, followed by k1), the RMS has performed both calculations. The applicant proposed to apply a single-first-order differential equation model (SFO) with the statistical software ”R”, while the RMS applied the solution function of the SFO kinetic (in principle both methods can be applied). These results are in agreement with the applicants calculation (TRR: BCF_k = 1316 and 1431 $L/kg_{wet\ fish}$; parent concentration: BCF_k = 1604 and 1745 $L/kg_{wet\ fish}$ for “sequential” or “sim-

ultaneous" calculation method).

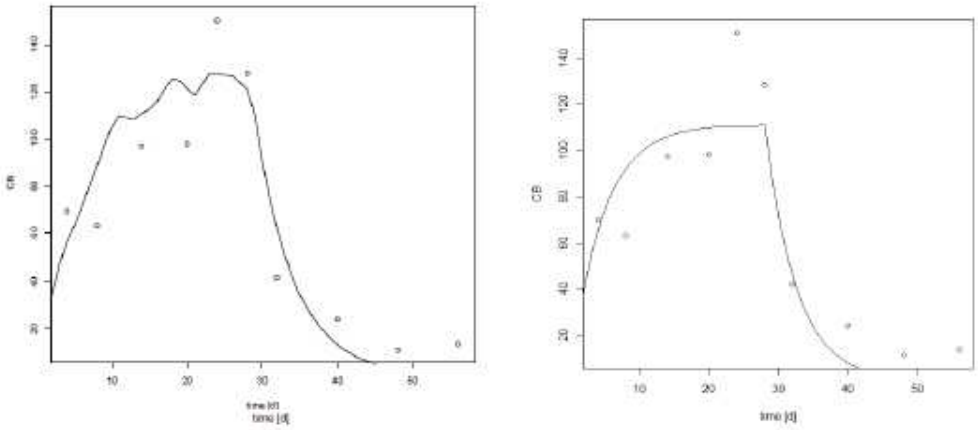
In addition to these fitting, the chi² error of these fitting methods was calculated. According to the "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" the chi² error allows to assess the quality of a fit, but both calculation methods showed a similar chi² error (22.3 for TRR and 25.9 for parent concentration). However, the same guidance states that a chi² error exceeding 15 is implying an inappropriate fit, the kinetic model SFO therefore does not seem to be adequate for the given data set. One reason for this are the two phases of the uptake phase with a plateau between days 4 and 8, the other reason is the accidental peak at day 22 in the water phase.
We conclude that either SFO is an inappropriate model or the quality of the data is insufficient for demonstrating SFO the correct model.
However, current guidance for the determination of BCF_k, including OECD TG 305, only considers first order kinetics and we therefore propose to stick with the given models and their limits.

A visual inspection of the kinetic fit with simultaneous estimation of k1 and k2 revealed that the fit seems to overestimate both the uptake and the depuration phase. The error of the fit does not seem to change the BCF in one certain direction (e.g. underestimation or overestimation) but could be compensated instead. Therefore we propose to accept the SFO model (until better methods become available).

TRR		
Parameter	**RMS (k1 und k2 simultan geschätzt, S.58)**	**RMS (k1 und k2 sequentiell geschätzt, S.56 ff)**
k1	217,5	289,5
k2	0,152	0,22
BCF	1430,921053	1315,909091
chi²-error	22,3	22,3



parent concentration		
Parameter	**RMS (k1 und k2 simultan geschätzt, S.58)**	**RMS (k1 und k2 sequentiell geschätzt, S.56 ff)**
k1	265,3	352,9
k2	0,152	0,22
BCF	1745,394737	1604,090909
chi²-error	25,9	25,9



The evaluation presented above is based on lipid-normalised values. Based on kinetic modelling and parent concentration, a $BCF_{\mathbf{k}}$ = 1745 L/kg can be derived simultaneous estimation of k1 and k2, resulting in a BCF_k of 1745 $L/kg_{wet\ fish}$). Applying growth-correction results in $BCF_{\mathbf{k}}$ = 1822 $L/kg_{wet\ fish}$
.

III. CONCLUSION

The bioconcentration potential of beta-cyfluthrin was investigated in bluegill sunfish at an average exposure concentration of 0.12 µg eq/L. The plateau level, determined as the average concentration in the fish of the last two time intervals of the uptake phase, was 0.172 µg eq/g. After exposure, a quick depuration was observed.

The lipid-normalised steady-state BCF [L kg^{-1}] based on measured concentration of the parent substance* BCF_{SSL} (parent) is 2295 However, the calculation of the $BCF_{steady\ state}$ directly relies on the concentration of the test substance at the steady state in fish and water. It is not possible to determine this concentration at the steady state within the new study without a high level of uncertainty. Therefore, we propose to consider the $BCF_{steady\ state}$ as additional information only. The kinetic BCF_k does not depend on measured concentrations. Thus, it provides a more reliable result than the $BCF_{steady\ state}$. The lipid- and growth corrected kinetic BCF is 1822.

B.9.2.3 Potential for endocrine disruption

Effects of cyfluthrin on fish were studied in a flow-through early life-stage test (ELS, 58 days) with rainbow trout and in a flow-through full life cycle test (FLC, 307 days) with fathead minnow.
The NOEC of 0.01 µg/L (adjusted to beta-cylfuthrin 0.0042 µg/L) in the fish ELS study was found for the parameters growth and fish showing behavioural signs.
The NOEC of 0.14 µg/L (adjusted 0.0059 µg/L) in the fish FLC study was based on effects on the parameters survival (parental and F1 generation) and hatching success (F1 generation) observed at the LOEC of 0.29 µg/L (adjusted 0.12 µg/L). No statistically significant effects on weight or length nor effects on reproductive success, as measured number of spawns, number of eggs, number of eggs/spawn, number of reproductive days and the number of eggs/pair/reproductive day were observed up to the highest concentration tested (0.29 µg/L/adjusted 0.12 µg/L).
Based on the absence of any indication of relevant effects to fish up to 0.14 µg/L (adjusted 0.0059 µg/L) in the laboratory it is concluded that beta-cyfluthrin has no potential endocrine disrupting properties. Moreover, the comprehensive data on mammalian toxicology do not give any indication. Therefore, no further testing for endocrine properties is required.
As part of US EPA's Endocrine Disruption Screening program (EDSP), however, a battery of Tier 1 screening assays exist on cyfluthrin. The studies support the conclusion that cyfluthrin does not interact with estrogen, androgen or thyroid systems to cause adverse effects. These studies were conducted specifically on cyfluthrin to fulfil national US EPA requirements and should not be taken into consideration as part of the Annex I Renewal process on beta-cyfluthrin as the existing data package on beta-cyfluthrin/cyfluthrin does not trigger any new studies to be conducted to further assess the endocrine toxicity of beta-cyfluthrin.

B.9.2.3.1 Acute toxicity to aquatic invertebrates

The current Annex I listing of beta-cyfluthrin is based on the studies of Forbis (1994) and Heimbach (1988). However, the latter study did not include an analytical verification of the test concentrations and the study of Forbis (1994) showed no clear dose/response and the EC50 was at the lower end of the test range. Therefore, a new acute study with *Daphnia magna* was conducted and is summarised below (Kimmel, 2014a).

To adjust the endpoints obtained in tests performed with cyfluthrin to beta-cyfluthrin, they have to be multiplied with a factor of 0.42 due to the lower content of the active (fish toxic) diasteromer pairs II and IV in cyfluthrin (see also 0)
Although there is evidence of racemisation in water and thus, a partial conversion from diastereomer pair II to I and IV to III from the mesocosm studies of Heimbach 1989 and 1990, this does not generally justify the assumption that beta-cyfluthrin converts into the less toxic cyfluthrin when sprayed into water bodies. Likewise, raw data from the hydrolysis study by Krohn 1997a (see Volume_3CA-B-8.2.1) do not exclude a slight epimerisation process in general, but do not provide clear evidence for epimerisation. Due to the study design it is not possible to distinguish between the processes of hydrolysis and epimerisation.
The reaction pathway of the racemisation of the closely related pyrethroid cypermethrin is well described in Nillos et al. 2009. According to this publication, the reaction is due to a positive partial charge at the α-C atom. Therefore, this carbon is relatively acidic leading to an easy loss of the C-

bound proton and consequently creating an anion. The reprotonation can occur from both sides. However, Nillos et al. (2009) used alcoholic (protic) solvents being responsible for reprotonation and stated that the epimerisation was not observed in pure water. Moreover, the authors cited Perschke and Hussain (1992) reporting that the addition of HCl prevented the isomerisation in case of deltamethrin. Overall, it is not clear which further components are needed to support this racemisation. Furthermore it appears quitelikely that an alkaline pH favours the racemisation since the separation of the proton from the α-C atom is supported by more alkaline conditions.
The mentioned mesocosm studies Heimbach 1989 and 1990 showed slight to moderate alkaline conditions. Therefore, no conclusion can be drawn for the fate of isomers in neutral or slightly acidic water bodies.

Concerning aquatic metabolites, reference is made to the acute studies on which Annex I inclusion of beta-cyfluthrin was based. In addition, a new study with *Daphnia magna* and the metabolite FPB-acid is available and summarised below.

Table B.9.2-10: Acute toxicity beta-cyfluthrin and the metabolites FPB-acid, DCVA and FPB-aldehyde to aquatic invertebrates

Species	Test design	EC50 (µg as/L)	NOEC (µg as/L)	Reference	reliability
Beta-Cyfluthrin					
Daphnia magna	48 h flow-through	0.290 (mm)	< 0.200 (mm)	KIIA 8.3.1.1/01 98515 Forbis, 1994 M-056226-01-1 R-19103	valid, but not plausible When taking into account the described sublethal effects: Quiescent, trapped on the bottom and clumping of daphnids (signs of immobilisation) the true EC50 < 0.2 µg/L (the lowest real concentration tested)
Daphnia magna	48 h static	2.0 (nom)	0.1 (nom)	KIIA 8.3.1.1/02 HBF/DM 78 Heimbach, 1988 M-056188-01-2 R-19102	Not valid, no analytics
Daphnia magna	48 h semi-static	0.105 (mm) [0,077 – 0,14 µg/L]	0.05 (mm)	KIIA 8.3.1.1/03 D58707 Kimmel, 2014a M-481046	valid
Americamysis bahia	96 h flow-through	0.0022 (mm)	0.0013 (mm)	KIIA 8.3.1.3/01 106797 Machado, 1994a M-056044-01-1 R-34702	Valid

Americamysis bahia	96 h flow-through	0.0023 (mm)	0.00086 (mm)	KIIA 8.3.1.3/02 106588 Machado, 1994b M-056064	Valid, but bad spacing: 0,0015 µg/L → 5 % Mortality 0,0029 µg/L → 75 % mortality
Cyfluthrin					
Gammarus pulex	21 d/static conducted with the formulation Cyfluthrin EC 050	$NOEC_{behaviour}$ = 0.00043 (mm) Adjusted values		KIIA 8.3.1.3/05; KIIA 8.3.2.1/06 (KIIIA110.2.6/01) HBF/SP 01-99 Heimbach, 2000 M-020399-01-1 R-19104 Please refer to Volume 3CP_Bulldock EC 25_B-9.3.2 /KIIIA1 10.2.6/01	study part without sediment: plausible
Daphnia magna	21 d/static with sediment conducted with the formulation Cyfluthrin EC 050 Xylol	EC15 (2 d) = 0.13 µg as/L (nominal) EC50 (2 d) = 0.34 µg as/L (nominal)		KIIA 8.3.1.1/08; KIIA 8.3.2.1/05 Heimbach, 1999 M-041214-01-1 Please refer to Volume 3CP_Bulldock EC 25_B-9 KIIIA1 10.2.6/01	supplementary information
Americamysis bahia	96 h flow-through	0.0024 (mm)	0.0008 (mm)	KIIA 8.3.1.3/03 808 Surprenant, 1987 M-027941-01-1	Valid
Hyalella azteca	96 h flow-through	0.00055 adjusted to beta-cyfluthrin: 0.000231	-	KIIA 8.3.1.3/04 M-458228-01-1 Bradley, 2013	Valid
FPB-acid					
Daphnia magna	48 h	85000 (nom)	-	KIIA 8.3.1.1/06 Lit. 6002 Hill, 1989 M-090574-01-1 R-19095	Additional information
Daphnia magna	**48 h static**	**39300 (nom)**	**12500 (nom)**	KIIA8.2.4.8/07 09 EBFRL002 Bruns, 2010 M-363182-01-1 R-27963	Valid
DCVA					
Daphnia magna	**48 h static**	**25000 (nom)**	**5600 (nom)**	KIIA 8.3.1.1/04 505 Forbis and Burgess, 1984 M-034747-01-1 R-19099	Valid except for missing analytical data. DCVA is considered to be stable in the aquatic environment.

Daphnia magna	48 h static	130000 (nom)	-	KIIA 8.3.1.1/06 Lit. 6002 Hill, 1989 M-090574-01-1 R-19095	Additional information
FPB-aldehyde					
Daphnia magna	**48 h static**	**1300 (nom)**	**1000 (nom)**	KIIA 8.3.1.1/05 504 Forbis and Burgess, 1984 M-034810-01-1 R-19098	Valid except for missing analytical data.

Values in bold: Endpoints used for risk assessment
mm: mean measured
nom: nominal initial

B.9.2.3.2 Acute toxicity to Daphnia magna

KIIA 8.3.1.1/01

Author:	Forbis, A. D.
Title:	Acute toxicity of FCR 4545-1 (techn.) to *Daphnia magna* under flow through conditions
Date:	24 August 1994
Doc ID:	M-056226-01-1
Report no.:	98515
Guidelines:	US-EPA FIFRA § 72-2 guideline and OECD Guideline No. 202
GLP:	yes
Validity:	Valid, but no plausible results

Deviations: The study is valid according to the current OECD Guideline No. 202, but not plausible
Test material: beta-Cyfluthrin techn. (FCR 4545), purity: not specified, batch no. 79R29-46E
Results: The 48-hour EC_{50} value for *Daphnia magna* exposed to beta-cyfluthrin was 0.29 µg as/L (mean measured).
The no observed effect concentration was determined with less than < 0.20 µg as/L and the lowest observed- effect-concentration with 1.0 µg as/L based on mean measured concentrations. At all test concentrations more or less immobile *Daphnia* were observed on the bottom.
Conclusion:
EC_{50} (48 h) < 0.20 µg as/L
When taking into account the described sublethal effects:
Quiescent, trapped on the bottom and clumping of daphnids (signs of immobilisation) the true EC_{50} < 0.2 µg/L (the lowest real concentration tested)

KIIA 8.3.1.1/02

Author:	Heimbach, F.
Title:	Acute toxicity of FCR 4545 (techn.) to water fleas
Date:	22 January 1988
Doc ID:	M-056188-01-2
Report no.:	HBF/DM 78
Guidelines:	US-EPA FIFRA § 72-2 guideline and OECD Guideline No. 202

GLP:	yes
Validity:	Not valid, no analytical data

Guideline: OECD Guideline No. 202
Deviations:
Except for lacking analytical verification of the test concentrations, the study is valid according to the current OECD Guideline No. 202.
Test material: beta-Cyfluthrin techn. (FCR 4545), purity: 98.3 %, batch no. 16001/87-46E
Results: As the EC_{50} falls in the range of solubility in water, an exact value for an EC cannot be stated; the EC_{50} (48 hours) for *Daphnia magna* is about 2.0 µg as/L based on nominal concentrations. The 'no-observed-effect-concentration' (NOEC) (48 hours) was 0.1 µg as/L based on nominal concentrations.
Conclusion:
Based on nominal values the EC_{50} (48 h) =2.0 µg as/L.
Supplemental information

KIIA 8.3.1.1/03 (newly submitted with renewal dossier)

Author:	Kimmel, S.
Title:	Beta-Cyfluthrin: Acute toxicity to *Daphnia magna* in a 48- Hour Immobilisation Test
Date:	19 March 2014
Doc ID:	M-481046-01-1
Report no.:	D58707
Edition no.:	M-481046-01-1
Guidelines:	OECD Guideline No. 202
GLP:	yes
Validity:	yes

Deviations: None
Dates of experimental work: 25 September 2012 to 07 March 2013

Executive Summary
The acute toxicity of beta-cyfluthrin to *Daphnia magna* was determined in a 48-hour semi-static test. Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 0.01, 0.032, 0.1, 0.32 and 1.0 µg as/L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to a test water control (without test item) and a solvent control (60 µL DMF/L).
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. The measured concentrations of beta-cyfluthrin in the freshly prepared test media were between 40 and 75 % of nominal. The measured concentrations in the aged test medium at the end of the renewal periods were between 37 and 71 % of nominal. Therefore, all results are based on mean measured concentrations.
All validity criteria according to the guideline OECD 202 were fulfilled. However, as the effects at 0.05 µg/L (mm) and 0.16 µg/L (mm) increase from 0 %to 85 % the chosen spacing of test concentrations is regarded to be less appropriate.

Conclusion:
The 48-h EC_{50} for *Daphnia magna* exposed to beta-cyfluthrin based on mean measured concentration was 0.105 µg/L with a 95 % confidence interval of 0.077 to 0.14 µg/L. No effect on immobilisation was seen up to 0.05 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	**Beta-cyfluthrin**
Lot/Batch #:	PNBC000623
Purity:	99.3 % w/w

2. Vehicle and/or positive control: N,N-Dimethylformamide (DMF)

3. Test organisms:

Species:	Daphnia magna
Age:	First instars (6-24 h old)
Source:	Laboratory bred
Loading:	5 organisms per vessel (100 mL glass beakers containing 50 mL test solution)

4. Environmental conditions:

Temperature:	20 to 21 C°
Photoperiod:	Light/dark 16/8 h
Light intensity:	400 to 540 lux
pH: Start of the test:	7.8
End of the test:	7.7 to 7.8
Dissolved oxygen:	Start of the test: 8.4 mg O_2/L End of the test: 8.2-8.4 mg O2/L
Hardness:	250 mg/L CaCO3

B. STUDY DESIGN

1. Experimental treatments

The effects of Beta-cyfluthrin on *Daphnia magna* were evaluated in a 48-hour semi-static toxicity test (renewal after 24 hours). Twenty *Daphnia* (4 replicates of 5 animals per test beaker) per concentration were exposed to 0.01, 0.032, 0.1, 0.32 and 1.0 µg as/L nominal concentrations. In addition, 4 x 5 *Daphnia* were exposed to a test water control (without test item) and a solvent (60 µL DMF/L) control. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations

Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from the control and from the test concentrations 0.1, 0.32 and 1.0 µg/L at the beginning and at the end of the test.

3. Statistical calculations

The 24- and 48-hour EC50 and the 95 % confidence limits were calculated as far as possible by Moving Average Interpolation.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on measured concentrations of beta-cyfluthrin, the following EC50 values for immobilisation after 24 and 48 hours of semi-static exposure were assessed.

Timepoint	EC_{50} [µg as/L, mean measured]	lower 95 % cl [µg as/L, mean measured]	upper 95 % cl [µg as/L, mean measured]
24 hours	> 0.62	n.d.	n.d.
48 hours	0.105	0.077	0.14

n.d not determined

The 48-hour NOEC was 0.05 µg as/L and the 48-hour EC_{100} was > 0.62 µg as/L.

Analytical data
In the freshly prepared test solutions at test initiation revealed recoveries ranged between 59 – 75 % (0 d- at start) and 40 % - 65 % (test medium renewal after 24 h) of the corresponding nominal concentrations.
The corresponding concentrations of the aged test solutions after 24 hours exposure period ranged between 37 % and 55 % of nominal values and after 48 hours exposure period between 53 and 71 %. Therefore, all results are based on mean measured test item concentrations.
However, as the measured concentrations in the fresh test medium (24 h) were slightly lower than in the aged test medium analytic data are not completely comprehensible.

B. OBSERVATIONS

After 48 hours of exposure, no immobilised test organisms were determined in the controls and up to and including the test item concentration of 0.1 µg as/L. At the next higher concentration of 0.32 µg as/L, 85 % of the daphnids were found to be immobile. At the highest test concentration of 1.0 µg/L, 90 % of the test organisms were found to be immobile. The measured values for these physical chemical parameters met the required range and yielded no deviation from guideline recommendations.

Table B.9.2-11: Effect of beta-cyfluthrin on the mobility of *Daphnia magna*

Nominal test concentration [µg as/L]	Mean measured concentration [µg as/L]	Number of exposed Daphnia per replicate	Number of immobile Daphnia after 24 hours		Number of immobile Daphnia after 48 hours	
			n	%	n	%
Control	-	20	0	0	0	0
Solvent control	-	20	0	0	0	0
0.01	n.a.	20	0	0	0	0
0.032	n.a.	20	0	0	0	0
0.1	0.05	20	0	0	0	0
0.32	0.16	20		0	17(2E+F 1A)	85
1.0	0.62	20	2(13F, 3A)	10	18 (2D+F)	90

n.a.: not analysed since below NOEC of the study
Values in parenthesis: number of test animals with adverse effects:
A: daphnids trapped at the water surface
D: daphnids discolored/pale
F: reduced swimming activity

The test is considered to be valid, as in the control and the solvent control no daphnids showed immobilisation or other signs of disease or stress (e.g., discoloration or unusual behavior such as trapping at the surface water). Furthermore, the dissolved oxygen concentration at the end of the test was ≥3 mg/L in the control and test vessels.

III. CONCLUSIONS

The 48-h EC_{50} for *Daphnia magna* exposed to beta-cyfluthrin based on measured concentration was 0.105 µg/L with a 95 % confidence interval of 0.077 to 0.14 µg/L. No effect on immobilisa-

tion was seen up to 0.05 µg/L (mm).
However, as the effects at 0.05 µg/L (mm) and 0.16 µg/L (mm) increase from 0 % to 85 % the chosen spacing of test concentrations is regarded to be less appropriate.

KIIA 8.3.1.1/04

Author:	Forbis, A. D.; Burgess, D.
Title:	Acute toxicity of DCVA to *Daphnia magna*
Date:	25 June 1984
Doc ID:	M-034747-01-1
Report no.:	505
Guidelines:	US-EPA FIFRA
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 202. No analytic on the test substance was conducted. However, DCVA is considered to be stable in the aquatic environment. (see Volume_3CA_B-8.2.2).

Test material: DCVA (Dichlorovinylcarboxylic acid), purity: 99.9 %, batch no. 150-6-52

Results: The 48 hour EC_{50} was 25000 µg/L (nominal). The no effect level observed for DCVA was 5600 µg/L (nominal) after 48 hours, which was based on the lack of mortality and abnormal effects.

Conclusion: EC_{50} (48 h) = 25000 µg/L; NOEC (48 h) =5600 µg/L

KIIA 8.3.1.1/05

Author:	Forbis, A. D.; Burgess, D.
Title:	Acute toxicity of FPB ald to *Daphnia magna*
Date:	25 June 1984
Doc ID:	M-034810-01-1
Report no.:	504
Guidelines:	US-EPA FIFRA
GLP:	yes
Validity:	valid

Guideline: US-EPA FIFRA
Deviations: Except for lacking analytical verification of the test concentrations, the study is valid according to the current OECD Guideline No. 202.
Test material: FPB-aldehyde **(**Fluorphenoxybenzaldehyde), purity: 96.2 %, batch no. 150-6-51

Results: The 48 hour EC_{50} was 1300 µg/L (nominal). The no effect level observed for FPB-aldehyde was 1000 µg/L (nominal) after 48 hours, which was based on the lack of mortality and abnormal effects.
Conclusion:
EC_{50} (48 h) = 1300 µg/L; NOEC (48 h) = 1000 µg/L

KIIA 8.3.1.1/06

Author:	Hill, I. R.
Title:	Aquatic organisms and pyrethroids
Date:	1990
Doc ID:	M-090574-01-1
Report no.:	Lit. 6002
Edition no.:	-
Guidelines:	Publication: Society of Chemical Industry, Great Britain, Journal: Pesticide Science, Volume:27, Pages:429-457, Year:1990
GLP:	no
Validity:	supplemental

Results: The FPB-acid 48 h EC_{50} for *Daphnia* is reported to be 85000 µg/L (nominal).
The DCVA 48 h EC_{50} for *Daphnia* is reported to be 130000 µg/L (nominal).
Conclusion: Supplemental information.

KIIA 8.3.1.1/07 (newly submitted with renewal dossier)

Author:	Bruns, E.
Title:	Acute toxicity of beta-Cyfluthrin FPB-acid (tech.) to the waterflea *Daphnia magna* in a static laboratory test system
Date:	1 February 2010
Doc ID:	M-363182-01-1
Guidelines:	OECD Guideline No. 202, (2004), U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982), EEC Directive 92/69/EEC, part C.2 (1992), OPPTS Guideline 850.1010 Draft (1996), modified, JMAFF 12 Nousan No. 8147 (2000)
GLP:	yes
Validity:	yes

Deviations: None

Executive Summary

The effects of FPB-acid on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Thirty *Daphnia* (6 replicates of 5 animals per test beaker) per concentration were exposed to 6.25, 12.5, 25, 50 and 100 mg/L FPB-acid (nominal concentrations). In addition, 6 x 5 *Daphnia* were exposed to test water without test substance (blank control). Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test. The analysed test concentrations ranged between 104 % and 108 % at the start and between 106 % and 111 % at the end of the test of the nominal values. Therefore, the results reported are related to nominal concentrations of the test item. All validity criteria according to the guideline OECD 202 were fulfilled.

The 48-h EC_{50} for *Daphnia magna* exposed to FPB-acid based on nominal concentration was 39.3 mg/L with a 95 % confidence interval of 28.1 to 55.1 mg/L. No effect on immobilisation was seen up to 12.5 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin FPB-acid
Lot/Batch #:	SES 10570-2-1
Purity:	99.2 % w/w

2. Vehicle and/or positive control: fully defined, artificial water

3. Test organisms:

Species:	*Daphnia magna*
Age:	First instars (< 24 h old)
Source:	Laboratory bred
Loading:	5 organisms per vessel (100 mL glass beakers containing 50 mL test solution)

4. Environmental conditions:

Temperature:	20.5 to 21.5 C°
Photoperiod:	Light/dark 16/8 h
pH:	Start of the test: 6.4-7.9
End of the test:	7.4-7.9
Dissolved oxygen:	Start of the test: 8.0-8.2 mg O2/L
End of the test:	8.3-8.4 mg O2/L
Conductivity:	532.0 µS/cm
Hardness:	231 mg/L CaCO3

B. STUDY DESIGN

1. Experimental treatments
The effects of FPB-acid on *Daphnia magna* were evaluated in a 48-hour static toxicity test. Thirty *Daphnia* (6 replicates of 5 animals per test beaker) per concentration were exposed to 6.25, 12.5, 25, 50 and 100 mg/L FPB-acid nominal concentrations. In addition, 6 x 5 *Daphnia* were exposed to test water without test substance (negative control). The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 48 hours.

2. Observations
Daphnids were observed for immobilisation at 24 and 48 hours and were not fed during the test.pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and at the end of the test. The water temperature in the test media was measured at the start of the test, 24, and 48 hours thereafter. Samples for the determination of the concentrations of FPB-acid in the test medium were taken from the control and from all test concentrations at the beginning and at the end of the test.

3. Statistical calculations
For EC50 determination, a dose response relationship curve (displayed as sigmoid, shaped over the logarithm of the concentration) was modelled by Probit Analysis (simple-linear regression) including computation of EC_{50} and 95 % confidence limits for immobility rates.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on nominal concentrations of FPB-acid, the following EC_{50} values for immobilisation after 24 and 48 hours of static exposure were determined.

FBP-acid			
Timepoint	**EC_{50} [µg as/L, mean measured]**	**lower 95 % cl [µg as/L, mean measured]**	**upper 95 % cl [µg as/L, mean measured]**
24 hours	> 0.62	n.d.	n.d.
48 hours	0.105	0.077	0.14

Analytical data: In the freshly prepared test solutions at test initiation revealed recoveries ranged between 104 % and 108 % (mean: 105 %) of the corresponding nominal concentrations.
The corresponding concentrations of the aged test solutions at the end of the 48 hours exposure period ranged between 106 % and 111 % (mean: 109 %) of nominal. No contaminations of FPB-acid were detected in samples from untreated water control. Accordingly, all results are related to nominal test concentrations of FPB-acid.

Reference item: For quality control of the breeding stock, an acute non-GLP toxicity test was performed separately in August 2009 using the reference substance $K_2Cr_2O_7$, p.a. grade (test concentrations: 0.56, 0.75, 1.00, 1.33 and 1.78 mg/L).
The 24 hour EC_{50} of 0.72 mg/L, as determined in this test, meets the range defined by OECD 202 (0.6 mg/L - 2.1 mg/L).

B. OBSERVATIONS

No immobilities or other effects on behaviour occurred in untreated control and in the 6.25 and 12.5 mg/L FPB-acid treatments within 48 hours of exposure. The immobilisation increases with increasing test concentration. At 100 mg/L FPB-acid, all daphnids are immobilised after 48 hours of exposure. The measured values for these physical chemical parameters met the required range and yielded no deviation from guideline recommendations.

Table B.9.2-12: Effects of FPB-acid to *Daphnia magna* (based on nominal concentrations)

Nominal test concentration [µg as/L]	Number of exposed Daphnia per replicate	Number of immobile Daphnia after 24 hours		Number of immobile Daphnia after 48 hours	
		n	%	n	%
Control	30	0	0	0	0
6.25	30	0	0	0	0
12.5	30	0	0	0	0
25	30	2	6.7	2	6.7
50	30	7	23.3	18	60
100	30	30	100	30	100

All validity criteria according to the OECD 202 were fulfilled, as no immobility of daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

III. CONCLUSIONS

The 48-h EC_{50} for *Daphnia magna* exposed to FPB-acid based on nominal concentration was 39.3 mg/L with a 95 % confidence interval of 28.1 to 55.1 mg/L. No effect on immobilisation was seen up to 12.5 mg/L.

B.9.2.3.3 Acute toxicity to an additional aquatic invertebrate species

Acute studies with *Americamysis bahia* (formerly *Mysidopsis bahia*) were conducted for the US registration of beta-cyfluthrin and cyfluthrin. While the study with cyfluthrin was already evaluated for the current Annex I listing of beta-cyfluthrin, the studies conducted with beta-cyfluthrin were not previously peer-reviewed on EU level. Detailed study summaries are provided below. In addition, an acute study with the amphipod *Hyalella azteca* was conducted by the Pyrethroid Working Group for the US.

KIIA 8.3.1.3/01 (newly submitted with renewal dossier)

Author:	Machado, M.W.
Title:	Acute toxicity of FCR 4545 to the Mysid Shrimp (*Mysidopsis bahia*) Under Flow Through Conditions
Date:	17 October 1994
Doc ID:	M-056044-01-1
Report no.:	106797
Guidelines:	US-EPA FIFRA § 72-3 guideline
GLP:	yes
Validity:	valid

Deviations: None

Executive Summary

The effects of beta-cyfluthrin (FCR 4545, 14C-labelled) on *Mysidopsis bahia* were evaluated in a 96-hour flow-through toxicity test. Twenty shrimps (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.65, 1.1, 1.8, 3.0 and 5.0 ng/L beta-cyfluthrin nominal concentrations. In addition, 2 × 10 mysid shrimps were exposed to test water without test substance (blank control) and to a solvent control. Shrimps were observed for mortality and sublethal effects at test initiation and after 24, 48, 72 and 96 hours.
The analysed test concentrations ranged between 72 % and 96 % of the nominal concentrations (mean of replicates and 0-hour and 96-hour analysis). Therefore, the results reported are related to mean measured test concentrations, i.e. 0.61, 0.96, 1.3, 2.3 and 3.8 ng/L beta-cyfluthrin.
All validity criteria according to US EPA FIFRA Guideline 72-3 were fulfilled.

The 96-h LC_{50} for *Americamysis bahia* exposed to beta-cyfluthrin based on mean measured concentration was 2.2 ng/L with a 95 % confidence interval of 1.9 to 2.7 ng/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: [phenyl-U-14C]beta-Cyfluthrin (FCR 4545)
Lot/Batch #: C-652A
Radiopurity: > 98 %
Specific Activity: 56.7 mCi/mmol

2. Vehicle and/or positive control:

Test water: Seawater collected from Cape Cod Canal, Bourne, Massachusetts, USA
Solvent: Acetone

3. Test organisms:

Species:	*Americamysis bahia*
Source:	Laboratory bred (Lot# 94A45a), purchased from commercial supplier, FT. Collins, Colorado
Loading:	10 organisms per vessel

4. Environmental conditions:

Temperature:	21 to 22 °C
Photoperiod:	Light/dark 16/8 hours
Light intensity:	290 to 970 lux
pH:	Start of the test: 8.0
	End of the test: 7.8 - 7.9
Dissolved oxygen:	Start of the test: 6.1 – 6.9 mg O2/L
End of the test:	5.9 – 6.7 mg O2/L
Salinity:	32‰

B. STUDY DESIGN

1. Experimental treatments

The effects of beta-cyfluthrin on *Mysidopsis bahia* were evaluated in a 96-hour flow-through toxicity test using 14C-labelled test item. Twenty shrimps (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.65, 1.1, 1.8, 3.0 and 5.0 ng/L nominal concentrations. In addition, 2 × 10 mysid shrimps were exposed to test water without test substance (blank control) and to a solvent control.

2. Observations

Shrimps were observed for mortality and sublethal effects at test initiation and after 24, 48, 72 and 96 hours. Live brine shrimp nauplii (*Artemia salina*) were added to each test vessel containing live test organisms twice daily *ad libitum*. Dissolved oxygen concentrations, pH, salinity and temperature were measured once daily in both replicates of each treatment level and the controls. In addition, the temperature was continuously monitored in one replicate. Samples for the determination of beta-cyfluthrin in the test medium were taken from both replicates of the blank control and the test concentrations before test start and at test start and after 96 hours from each replicate of each treatment level.
All samples were extracted and analysed for [^{14}C]FCR 4545 using a liquid scintillation counting (LSC) procedure according. In addition to the exposure solution analyses, thin layer chromatography (TLC) was used to determine the radiopurity of the high test concentration (5.0 ng/L), the primary radiolabeled stock solution and the diluter stock solution.

3. Statistical calculations

The 96-hour LC50 value was determined by probit analysis including 95 % confidence limits.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on mean measured concentrations of [^{14}C]beta-cyfluthrin, the following LC_{50} values for mortality after 96 hours of flow-through exposure were assessed.

[^{14}C]beta-Cyfluthrin			
Timepoint	**value [ng as/L, mean measured]**	**lower 95 % cl [µg as/L, mean measured]**	**upper 95 % cl [µg as/L, mean measured]**
96-hour LC_{50}	2.2	1.9	2.7
96-hour NOEC	1.3	-	-

Analytical data: The analysed test concentrations of [^{14}C]beta-cyfluthrin ranged between 72 – 96 % of nominal treatment levels. The mean measured test concentrations were 0.61, 0.96, 1.3, 2.3 and 3.8 ng/L.

B. OBSERVATIONS

Mortality started at 1.3 ng /L beta-cyfluthrin (mean measured concentrations). In addition, sublethal effects (i.e., loss of equilibrium, lethargy) were observed among surviving mysids exposed to the 2.3 and 3.8 ng/L treatment levels.

Table B.9.2-13: Toxicity of [^{14}C]beta-cyfluthrin to *Americamysis bahia*

Nominal test concentration [ng as/L]	Mean measured concentration [ng as/L]	Number of exposed mysid shrimp per replicate	Cumulative mean Mortality [%]			
			24-hours	48-hours	72-hours	96-hours
Control	-	20	0	0	0	0
Solvent control	-	20	0	0	0	0
0.64	0.61	20	0	0	0	0
1.1	0.96	20	0	0	0	0
1.8	1.3	20	0	0	0	0
3.0	2.3	20	10 ^{BCD}	25 ^{BFGH}	45 ^{BK}	55 ^{BEL}
5.0	3.8	20	20 ^{AB}	65 ^{BE}	85 ^{DIJ}	90 ^{DJ}

All validity criteria according to the FIFRA Guideline 72-3 were fulfilled, as less than 10 % mortality of mysid shrimps was observed in control groups.

III. CONCLUSIONS

The 96-h LC_{50} for *Americamysis bahia* exposed to [^{14}C] beta-cyfluthrin based on mean measured concentration was 2.2 ng/L with a 95 % confidence interval of 1.9 to 2.7 ng/L.

KIIA 8.3.1.3/02 (newly submitted with renewal dossier)

Author:	Machado, M.W.
Title:	Acute toxicity of FCR 4545 to the Mysid Shrimp (*Mysidopsis bahia*) Under Flow Through Conditions
Date:	19 July 1994
Doc ID:	
Report no.:	106588
Edition no.:	M-056064-01-1 (R-34703)
Guidelines:	OCSPP Draft Guideline 850.1020
GLP:	yes
Validity:	valid

Deviations: None

Executive Summary

The effects of beta-cyfluthrin (FCR 4545, 14C-labelled) on *Mysidopsis bahia* were evaluated in a 96-hour flow-through toxicity test. Twenty shrimps (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.65, 1.1, 1.8, 3.0 and 5.0 ng /L nominal concentrations. In addition, 2 × 10 mysid shrimps were exposed to test water without test substance (blank control) and to a solvent control. Shrimps were observed for mortality and sublethal effects at test initiation and after 24, 48, 72 and 96 hours.

The analysed test concentrations ranged between 48 % and 78 % of the nominal concentration (mean of

replicates and 0-hour and 96-hour analysis). Therefore, the results were based on mean measured

concentrations, i.e. 0.50, 0.55, 0.86, 1.5 and 2.9 ng /L beta-cyfluthrin.
All validity criteria according to US EPA FIFRA Guideline 72-3 were fulfilled.

The 96-h LC_{50} for *Americamysis bahia* exposed to [^{14}C]beta-cyfluthrin based on mean measured concentration was 2.3 ng/L with a 95 % confidence interval of 1.5 to 2.9 ng/L.
However, as mortality at 1.5 ng/L (mm) and 2.9 ng/L (mm) increases from 5 % to 85 % the chosen spacing of test concentrations is regarded to be less appropriate.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	[penyl-U-14C]beta-Cyfluthrin (FCR 4545)
Lot/Batch #:	C-652A
Radiopurity:	> 98 %
Specific Activity:	56.7 mCi/mmol

2. Vehicle and/or positive control:

Test water:	Seawater collected from Cape Cod Canal, Bourne, Massachusetts, USA
Solvent:	Acetone

3. Test organisms:

Species:	*Mysidopsis bahia*
Source:	Laboratory bred (Lot# 94A12), purchased from commercial supplier, FT. Collins, Colorado
Loading:	10 organisms per vessel

4. Environmental conditions:

Temperature:	21 to 23 °C
Photoperiod:	Light/dark 16/8 hours
Light intensity:	320 to 430 lux
pH:	Start of the test: 7.7 – 7.8
End of the test:	7.8
Dissolved oxygen:	Start of the test: 7.2 – 7.4 mg O2/L
End of the test:	6.2 – 6.6 mg O2/L
Salinity:	31 to 32‰

B. STUDY DESIGN

1. Experimental treatments

The effects of beta-cyfluthrin on *Americmysis bahia* were evaluated in a 96-hour flow-through toxicity test using 14C-labelled test item. Twenty shrimps (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.65, 1.1, 1.8, 3.0 and 5.0 ng/L nominal concentrations. In addition, 2 × 10 mysid shrimps were exposed to test water without test substance (blank control) and to a solvent control.

2. Observations

Shrimps were observed for mortality and sublethal effects at test initiation and after 24, 48, 72 and 96 hours. Live brine shrimp nauplii (*Artemia salina*) were added to each test vessel containing live test organisms twice daily *ad libitum*. Dissolved oxygen concentrations, pH, salinity and temperature were measured once daily in both replicates of each treatment level and the controls. In addition the temperature was continuously monitored in one replicate. Samples for the determination of the beta-cyfluthrin in the test medium were taken from both replicates of the blank control and the test concentrations before test start and at test start and after 96 hours from each replicate of each treatment level. All samples were extracted and analysed for [^{14}C]FCR 4545 using a liquid scintillation counting

(LSC) procedure according. In addition to the exposure solution analyses, thin layer chromatography (TLC) was used to determine the radiopurity of the high test concentration (5.0 ng/L), the primary radiolabeled stock solution and the diluter stock solution.

3. Statistical calculations
The 96-hour LC_{50} value was determined by nonlinear interpolation including 95 % confidence limits calculated by binomial probability.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on mean measured concentrations of [^{14}C] beta-cyfluthrin, the following LC_{50} values for mortality after 96 hours of flow-through exposure were assessed.

[14C]beta-Cyfluthrin			
Timepoint	**value [ng as/L, mean measured]**	**lower 95 % cl [ng as/L, mean measured]**	**upper 95 % cl [ng as/L, mean measured]**
96-hour LC_{50}	2.3	1.5	2.9
96-hour NOEC	0.86	-	-

Analytical data
The analysed test concentrations of [^{14}C] beta-cyfluthrin ranged between 31 – 61 % of nominal treatment levels (mean measured: 48 – 78 % of nominal). The mean measured test concentrations were 0.50, 0.55, 0.86, 1.5 and 2.9 ng/L.
Two out of four control samples and two out of four solvent control samples were contaminated. The contamination ranged from 0.087 to 0.40 ng/L.

B. OBSERVATIONS

Mortality increased at 2.9 ng beta-cyfluthrin/L of mean measured concentrations. Sublethal effects (i.e., loss of equilibrium, lethargy) were observed among surviving mysids exposed to the 1.5 and 2.9 ng/L treatment levels.

Table B.9.2-14: Toxicity of [^{14}C]beta-cyfluthrin to *Americamysis bahia*

Nominal test concentration [ng as/L]	Mean measured concentration [ng as/L]	Number of exposed mysid shrimp per replicate	**Cumulative mean Mortality [%]**			
			24-hours	**48-hours**	**72-hours**	**96-hours**
Control	-	20	0	0	0	0
Solvent control	-	20	0	0	0	0
0.64	0.50	20	0	0	0	0
1.1	0.55	20	0	0	0	0
1.8	0.86	20	0D	0D	0D	5
3.0	1.5	20	0AC	5AC	5A	5A
5.0	2.9	20	25 AB	40AEF	70 AG	75 AH

All validity criteria according to the FIFRA Guideline 72-3 were fulfilled, as less than 10 % mortality of mysid shrimps was observed in control groups.

III. CONCLUSIONS

The 96-h LC_{50} for *Americmysis bahia* exposed to [^{14}C] beta-cyfluthrin based on mean measured concentration was 2.3 ng/L with a 95 % confidence interval of 1.5 to 2.9 ng/L.
However, as mortality at 1.5 ng/L (mm) and 2.9 ng/L (mm) increases from 5 % to 85 % the chosen spacing of test concentrations is regarded to be less appropriate.

KIIA 8.3.1.3/03

Author:	Surprenant, D.C.
Title:	Acute toxicity of FCR 4545 to the Mysid Shrimp (*Mysidopsis bahia*) Under Flow Through Conditions
Date:	30 January 1987
Doc ID:	M-027941-01-1
Report no.:	BW-87-1-2277
Guidelines:	US-EPA FIFRA § 72-3 guideline
GLP:	yes
Validity:	valid

Deviations: None

Test material: Cyfluthrin techn. (Baythroid), purity: 97.4 %, batch no. 83-R-270-272

Results: The 96-h LC_{50} for *Mysidopsis bahia* exposed to cyfluthrin based on mean measured concentration was 2.46 ng/L with a 95 % confidence interval of 1.96 to 3.26 ng/L. The NOEC was 0.81 ng/L.
Conclusions:

LC_{50} (96 h) = 2.46 ng/L; NOEC (96 h) = 0.81 ng/L

KIIA 8.3.1.3/04 (newly submitted with renewal dossier)

Author:	Bradley, M., J.
Title:	Cyfluthrin - Acute Toxicity to Freshwater Amphipods (Hyalella azteca) Under Flow-Through Conditions
Date:	24 June 2013
Doc ID:	
Report no.:	13656.6168
Edition no.:	M-458228-01-1
Guidelines:	US-EPA FIFRA § 72-3 guideline
GLP:	yes
Validity:	valid

Deviations: None

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item: Cyfluthrin technical
Lot/Batch #: FHER904218
Radiopurity: 95.8 %

2. Vehicle and/or positive control:

Test water: laboratory well water

Solvent: Acetone

3. Test organisms:

Species: *Hyalella azteca* SMV Lot No. 011713, eight days old at test initition

Source:	Smithers Viscient culture
Loading:	10 organisms per vessel

4. Environmental conditions:

Temperature:	22 - 24 °C
Photoperiod:	Light/dark 16/8 hours
Light intensity:	100 to 500 lux
pH:	Start of the test: 7.2
	End of the test: 7.2 – 7.4
Dissolved oxygen:	Start of the test: 8.7 – 9.2 mg O_2/L
End of the test:	7.5 – 9.5 mg O_2/L

B. STUDY DESIGN

1. Experimental treatments

The effects of cyfluthrin on *Hyalella azteca* were evaluated in a 96-hour flow-through toxicity test. Twenty amphipods (2 replicates of 10 animals per test beaker) per concentration were exposed to 0.20, 0.40, 0.80, 1.6 and 3.2 ng as/L nominal concentrations. In addition, 2 × 10 amphipods were exposed to test water without test substance (blank control) and to a solvent control.

2. Observations

The number of dead Hyalella in each test vessel was recorded at test initiation and after 24, 48, 72 and 96 hours of exposure. Death was determined by gently agitating the test solution around those amphipods that appeared to be immobile. If no physical response was observed, immobile Hyalella were inspected within a pipette and closely examined for any subtle physical movements (e.g. slight movements of the appendages). If upon further inspection there was no observed movement, the Hyalella were considered dead. Additional cues in combination with immobility, such as discoloration and decay, were also used to determine mortality. Biological observations and observations of the physical characteristics of each replicate test solution were made and recorded at test initiation and after 24, 48, 72 and 96 hours of exposure.

Prior to the start of the definitive exposure, samples were removed from all treatment level, control and solvent control solutions and analysed for cyfluthrin concentrations. Results of the pretest analyses were used to judge whether sufficient quantities of cyfluthrin were being delivered to the test vessels and whether the appropriate test concentrations were being maintained in order to initiate the definitive exposure.

During the in-life phase of the definitive study, one sample was removed from each test, control and solvent control solution for analysis of cyfluthrin concentration at 0 hour (test initiation) and 96 hours (test termination). Samples were collected from the approximate midpoint of the test vessels by siphoning. Samples analysed at test initiation (0 hour) and test termination (96 hour) were a composite of both replicates of each test level, control and solvent control.

Exposure solutions and QC samples were analysed for cyfluthrin using gas chromatography with mass selective detection (GC/MSD).

3. Statistical calculations

A computer program, CETIS-Comprehensive Environmental Toxicity Information System™ (Ives, M., 2011), will be used to estimate LC50 values. An LC_{50} value cannot be calculated if the mortality data derived is insufficient. The method selected is determined by the data base (i.e., presence or absence of 100 % response, number of partial responses, etc.).

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on mean measured concentrations of [^{14}C]beta-cyfluthrin, the following LC_{50} values for mortality after 96 hours of flow-through exposure were assessed.

Based on mean measured concentrations of cyfluthrin technical, LC_{50} values for mortality after 96 hours of flow-through exposure were assessed.

Cyfluthrin			
Timepoint	**value [ng as/L, mean measured]**	**lower 95 % cl [ng as/L, mean measured]**	**upper 95 % cl [ng as/L, mean measured]**
96-hour LC_{50}	0.55	0.47	0.64

Analytical data: The analysed test concentrations of cyfluthrin ranged between 76 – 87 % of nominal treatment levels. The mean measured test concentrations were 0.17, 0.32, 0.66, 1.2 and 2.4 ng/L.

B. OBSERVATIONS

Mean measured concentrations, percent mortality, and observations recorded during the 96-hour definitive test are presented in Table 4. Following 96 hours of exposure, 10, 10, 70, 100 and 100 % mortality was observed among *Hyalella azteca* exposed to mean measured concentrations of 0.17, 0.32, 0.66, 1.2 and 2.6 ng/L, respectively. All surviving *Hyalella* exposed to the 0.66 ng/L treatment level were observed to be lethargic. Following 96 hours of exposure, 5 % mortality was observed among *Hyalella* exposed to the control while no mortality or adverse effects were observed among *Hyalella* exposed to the solvent control.

Table B.9.2-15: Toxicity of cyfluthrin to *Hyalella azteca*

Nominal test concentration [ng as/L]	**Mean measured concentration [ng as/L]**	**Number of exposed mysid shrimp per replicate**	**Cumulative mean Mortality [%]**			
			24-hours	**48-hours**	**72-hours**	**96-hours**
Control	-	20	0	0	5	5
Solvent control	-	20	0	0	0	0
0.20	0.17	20	0	5[a]	5	10
0.40	0.32	20	5	5	5	10
0.80	0.66	20	20[b]	45[ac]	65[ac]	70[b]
1.6	1.2	20	35[d]	50[d]	95[e]	100
3.2	2.4	20	50[d]	65[e]	95[e]	100

a Several *H. azteca* were observed to be lethargic.
b All surviving *H. azteca* were observed to be lethargic.
c Two *H. azteca* were observed to be immobilised.
d Several *H. azteca* were observed to be immobilised.
e All surviving *H. azteca* were observed to be immobilised.

III. CONCLUSIONS

The 96-h LC50 for *Hyalella azteca* exposed to cyfluthrin based on mean measured concentration was 0.55 ng/L with a 95 % confidence interval of 0.47 to 0.64 ng/L.

B.9.2.4 Long-term and chronic toxicity to aquatic invertebrates

The current Annex I listing of beta-cyfluthrin is based on studies with cyfluthrin (Heimbach, 1988 and Forbis *et al*., 1984). A new chronic study with *Daphnia magna* was conducted with beta-cyfluthrin and is summarised below (Kimmel, 2014b). In addition, a life-cycle study with *Americamysis bahia* was conducted for the US registration of beta-cyfluthrin (Schwader, 2013).

Table B.9.2-16: Chronic toxicity beta-cyfluthrin /cyfluthrin

Species	Test design	EC50 (µg as/L)	NOEC (µg as/L)	Reference	reliability
Beta-Cyfluthrin					
Daphnia magna	21 d semi-static	>0.057 (mm)	0.025 (mm)	KIIA 8.3.2.1/03 D58718 Kimmel, 2014 M-480965-01-1 R-30152	valid
Americamysis bahia	28 d flow-through	>0.0015 (mm)	0.00041 (mm)	KIIA 8.3.2.1/04 EBFRL028 M-465880-01-1 Schwader, 2013	valid
Cyfluthrin					
Daphnia magna	21 d semi static	-	0.100 (nom)	CA 8.2.5.1/01 HBF/RDM 01 Heimbach, 1988 M-008718-01-2 R-19106	Not valid
Daphnia magna	21 d flow-through	-	0.020 (mm)	CA 8.2.5.1/02 557 Forbis et al., 1984 M-025043-01-1 R-19105	valid
Gammarus pulex	21 d/static conducted with the formulation Cyfluthrin EC 050	EC_{50} (2 d) = 0.0075 (mm) EC_{50} (7 d) = 0.0021 (mm) EC_{50} (21d) =0.000378 (mm) $NOEC_{behaviour}$ = 0.000109 (mm) Adjusted values		KIIA 8.3.1.3/05, KIIA 8.3.2.1/06 (KIIIA110.2.6/01) HBF/SP 01-99 Heimbach, 2000 M-020399-01-1 R-19104 Please refer to Volume 3CP_Bulldock EC 25_B-9.3.2 /KIIIA1 10.2.6/01	study part without sediment: plausible
Daphnia magna	29 d/static/ with sediment/ conducted with the formulation Cyfluthrin EC 050 Xylol	EC15 (2 d) = 0.13 µg as/L (nominal) EC50 (2 d) = 0.34 µg as/L (nominal) NOEC (29 d) = 0.1mg as/L		KIIA 8.3.1.1/08, KIIA 8.3.2.1/05 Heimbach, 1999 M-041214-01-1 Please refer to Volume 3CP_Bulldock EC 25_B-9 KIIIA1 10.2.6/01	supplementary information

Values in bold: Endpoints used for risk assessment
mm: mean measured
nom: nominal initial

B.9.2.4.1 Reproductive and development toxicity to Daphnia magna

KIIA 8.3.2.1/01

Author:	Forbis, A. D. *et al.*
Title:	Chronic toxicity of 14C-Cyfluthrin to *Daphnia magna* under flow-through test conditions
Date:	7 November 1984
Doc ID:	M-025043-01-1
Report no.:	557
Guidelines:	OECD Guideline No. 211 and US-EPA FIFRA § 72-4 guideline
GLP:	yes
Validity:	valid

Guideline: OECD Guideline No. 211 and US-EPA FIFRA § 72-4 guideline
Deviations: The study is valid according to the current OECD Guideline No. 211
Test material: ^{14}C-Cyfluthrin techn., purity: 94.7 %, lot no. 82R-8256 available
The chronic effects of cyfluthrin on *Daphnia magna* were investigated with ^{14}C-labelled test item in a flow through system.

Results: Analysed concentrations were between 63 % and 100 % of nominal.
The 21-day no-observed-effect-concentration for *Daphnia magna* exposed to cyfluthrin was 0.02 µg/L based on the mean measured concentrations. Effects on the number of offspring and the body lengths of parent water fleas were seen at the end of the study at 0.041 µg/L (LOEC), based on mean measured).
Conclusion: NOEC (21 d) = 0.02 µg/L

KIIA 8.3.2.1/02

Author:	Heimbach, F
Title:	Influence of Cyfluthrin (techn.) on the reproduction rate of water fleas (*Daphnia magna*)
Date:	22 January 1988
Doc ID:	M-008718-01-2
Report no.:	HBF/RDM 01
Guidelines:	"Prolonged Toxicity Study with *Daphnia magna* (Inhibition of Reproduction)", EEC XI/681/86 of December 1986
GLP:	yes
Validity:	valid, not suitable to deduce a endpoint for reproduction

Deviations: The study is valid according to the current OECD Guideline No. 211. However, since 10 out of 20 organisms were selected for fertility testing. Animals were selected by observable formation of eggs in the dorsal brood pouch. This was reasoned by the need to select only females.
However, a lack of eggs in the dorsal brood pouch does not necessarily mean that they were male. It is also possible that due to an effect of the active substance the production of eggs was disturbed in some female individuals.
According to the the current OECD Guideline No. 211, only female individuals should be introduced. Therefore, the introduction of both sexes is not comprehensible and causes an unkown uncertainty in regared to the reproductive capacity of *Daphnia magna* when exposed to the active substance.
Hence, it is not possible to deduce reliable ecotoxicological endpoints concerning the effects of beta-cyfluthrin on the reproduction of *Daphnia magna*.
Test material: Cyfluthrin techn., purity: 94.1 %, batch no. 1601031
Study design and Methods: The effects of technical cyfluthrin were investigated over 21 days under

semi static conditions.
Results: The highest concentration tested without effects (no observed effect concentration, NOEC) for reproduction of *Daphnia magna* was 100 ng as/L (nominal) during the test period of 21 days. The lowest concentration tested with effects (LOEC) was 178 ng as/L (nominal).
Conclusion: supplemental information

KIIA 8.3.2.1/03

Author:	Kimmel, S.
Title:	Beta-cyfluthrin: Effect on Survival and Reproduction of Daphnia magna in a Semi-Static Test over Three Weeks
Date:	19 March 2014b
Doc ID:	M-480965-01-1
Report no.:	D58718
Guidelines:	OECD Guideline No. 211, *Daphnia magna* Reproduction Test, October 03, 2008, EU Commission Directive 92/69/EEC, part C.20, *Daphnia magna* Reproduction Test (2001), EU Commission Regulation (EC) No 440/2008, C.20: "*Daphnia magn*a Reproduction Test.
GLP:	yes
Validity:	valid

Deviations: None
Dates of experimental work: 27 November 2012 to 14 March 2013
Executive Summary
The effect of the test item beta-cyfluthrin on the survival and reproduction of *Daphnia magna* was investigated in a semi-static test over 21 days. Ten Daphnia (1 animals per test beaker) per concentrationwere exposed to 0.001, 0.0032, 0.010, 0.032 and 0.10 µg as/L nominal concentrations. In addition, 10 Daphnia were exposed to a test water control (without test item) and a solvent control (60 µL DMF/L).
Daphnids were observed for immobilisation and reproduction on Days 0-2, 5, 7, 9, 12, 14, 16, 19 and 21 and were fed daily during the test. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from the test concentrations 0.010, 0.032 and 0.10 µg as/L and from the solvent control of the first, second and last week of the test, i.e. on day 0, 7 and 16, respectively. Samples for the determination of the stability of beta-cyfluthrin were taken at the end of two test medium renewal periods of 48 hours (days 2 and 9) and at the end of one renewal period of 72 hours (day 19).
In the application solutions of each measured renewal period, the test item concentrations were between 96 % and 107 % of the nominal values throughout the sample period. Thus, the test item was stable in the application solutions throughout each of the renewal periods. The measured concentrations in the freshly prepared test media of the nominal concentrations of 0.010, 0.032 and 0.10 µg as/L were between 40 and 133 % of nominal values at the start of the test medium renewal periods. In the stability control samples without food particles and daphnids, the measured concentrations were between 23 and 109 % of the nominal values at the end of the test medium renewal periods of 48 to 72 hours. All validity criteria according to the guideline OECD 211 were fulfilled.

The highest mean measured concentration of beta-cyfluthrin tested without effects after the exposure period of 21 days (21-day NOEC) based on reproduction was 0.025 µg as/L.
The lowest concentration tested with effects (21-day LOEC) was determined to be 0.057 µg as/L (mean measured). The 21-d EC_{10}, EC_{20} and EC_{50} for *Daphnia magna* exposed to beta-cyfluthrin based on mean measured concentrations were 0.023, 0.041 and >0.057 µg/L, respectively and with 95 % confidence intervals of 0.0017 to 0.034 µg/L, 0.020 to >0.057 µg/L and 0.076 to >0.057 µg/L, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin
Lot/Batch #:	PNBC000623
Purity:	99.3 % w/w

2. Vehicle and/or positive control: N,N-Dimethylformamide (DMF)

3. Test organisms:

Species:	Daphnia magna
Age:	First instars (< 24 h old)
Source:	Laboratory bred
Loading:	1 organisms per vessel (100 mL glass beakers containing 80 mL test solution)

4. Environmental conditions:

Temperature:	20 to 21 C°
Photoperiod:	Light/dark 16/8 h
Light intensity:	400 to 540 lux
pH:	7.5 to 8.0
Dissolved oxygen:	7.9 to 9.0 mg O2/L
Hardness:	250 mg/L CaCO3
Alkalinity:	0.9 mmol/L

B. STUDY DESIGN

1. Experimental treatments

The effects of beta-cyfluthrin on immobilisation and reproduction of *Daphnia magna* were evaluated in a 21 days semi-static toxicity test. Ten *Daphnia* (1 animals per test beaker) per concentration were exposed to 0.001, 0.0032, 0.01, 0.032 and 0.1 µg as/L nominal concentrations.
In addition, ten *Daphnia* were exposed to a test water control (without test item) and a solvent (60 µL DMF/L) control. The *Daphnia* were randomly placed into the test beaker and exposed to the test item for 21 days. The test media of all treatments were renewed on days 2, 5, 7, 9, 12, 14, 16 and 19 of the test period. The test animals were fed daily with a food mixture containing a suspension of green algae of the species *Scenedesmus subspicatus* and a fish food suspension.

2. Observations

The test replicates were observed for immobility of adults on days 0-2 and thereafter on day 5, 7, 9, 12, 14, 16, 19 and 21 before renewal of the test media. On the same dates, the test replicates were observed for the number of living and dead offspring and for the presence of aborted eggs. The reproduction rate was calculated as the total number of living offspring produced per parent female surviving until the end of the test pH-values and dissolved oxygen concentrations were determined in the test media at the beginning and the end of each renewal period. The water temperature was measured in one of the control replicates at the same time. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from all test concentrations and from the solvent control of the first, second and last week of the test, i.e. on day 0, 7 and 16, respectively. Samples for the determination of the stability of beta-cyfluthrin were taken at the end of two test medium renewal periods of 48 hours (days 2 and 9) and at the end of one renewal period of 72 hours (day 19).

3. Statistical calculations

The mean reproduction rates of the daphnids at the test concentrations were compared to the pooled controls by multiple Williams t-tests. Additionally, the EC10, EC20 and EC50 for the inhibition of the reproduction rate after 21 days were calculated by Probit Analysis.

II. RESULTS AND DISCUSSION

A. FINDINGS

Based on the mean measured concentrations of beta-cyfluthrin, the following endpoints for reproduction of the test animals after 21 days of semi-static exposure were assessed.

Endpoints	Inhibition of reproduction rate (21 days, mean measured concentration)
EC10 [µg as/L] 95 % confidence interval	0.023 0.0017 – 0.034
EC20 [µg as/L] 95 % confidence interval	0.041 0.020 - > 0.057*
EC50 [µg as/L] 95 % confidence interval	> 0.057* 0.076 - > 0.057
NOEC [µg as/L]	0.025
LOEC [µg as/L]	0.057

* Extrapolated value, EC_{50} and upper confidence interval are greater than the highest concentration tested

Analytical data:
In the application solution samples, the measured test item concentrations ranged from 101 % to 107 % of nominal in the freshly prepared application solutions (measured on days -1, 5, 12 and 19), and from 96 % to 103 % in the aged application solutions (measured on days 2, 9 and 16). Thus, the test item was stable in the application solutions throughout each of the renewal periods. The measured concentrations in the freshly prepared test media of the nominal concentrations of 0.010, 0.032 and 0.10 µg as/L were between 40 and 133 % of nominal values at the start of the test medium renewal periods. In the stability control samples without food particles and daphnids, the measured concentrations were between 23 and 109 % of the nominal values at the end of the test medium renewal periods of 48 to 72 hours.

B. OBSERVATIONS

The survival of *Daphnia magna* after 21 days was reduced at the mean measured concentrations of 0.025 and 0.057 µg as/L, which was however, not statistically significant reduced compared to pooled controls. The time of the first brood was not affected by the test item up to the mean measured concentration of
0.025 µg as/L.
The mean reproduction rate of the daphnids in the solvent control was 120 ± 18.4 living offspring per surviving adult. The corresponding value in the control was 133 ± 6.7.
No significant effect on reproduction was determined up to and including the mean measured test concentration of 0.025 µg as/L (Williams t-test, one-sided smaller, $\alpha = 0.05$). At the highest concentration of 0.057 µg as/L (mean measured), the mean reproduction rate of surviving daphnids was statistically significantly reduced. At this concentration the mean reproduction rate was 92±19.4 living offspring per surviving adult (73 % compared to the pooled controls).
With the exception of the reported mortality and reduced reproduction rates, no visible abnormalities were observed at the test animals during the test.

	Solvent Control	**Control**	**Beta-Cyfluthrin nominal (and measured) concentration [µg as/L]**				
			0.0010 (n.a.)	0.0032 (n.a.)	0.010 (0.0059)	0.032 (0.025)	0.10 (0.057)
Mortality [%] after 21 days of exposure	10	0	0	0	0	30	30

Mean re-production rate (living offspring per surviving adult)	120.3	132.9	140.7	147.7	141.5	114.7	92.3*
Mean re-production rate in % of pooled conrols	100.0		110.8	116.5	111.4	90.3	72.7

Note: Both controls (water and solvent) were pooled for statistical analysis. Mean reproduction rates are referred to the results of the pooled controls.
* statistically significantly lower than the pooled controls value, results of a Williams t-test, one-sided smaller, $\alpha = 0.05$
n.a.: not analysed since below NOEC of the study.

The test is considered to be valid, as in the control and solvent control the survival of the parent animals at the end of the test was 100 % and 90 %, respectively. Furthermore, the mean number of live offspring produced per parent animal surviving at the end of the test is > 60 in the control and solvent control.

III. CONCLUSIONS

The highest mean measured concentration of beta-cyfluthrin tested without effects after the exposure period of 21 days (21-day NOEC) based on reproduction was 0.025 µg as/L.
The lowest concentration tested with effects (21-day LOEC) was determined to be 0.057 µg as/L (mean measured). The 21-d EC_{10}, EC_{20} and EC_{50} for *Daphnia magna* exposed to beta-cyfluthrin based on mean measured concentrations were 0.023, 0.041 and >0.057 µg/L, respectively and with 95 % confidence intervals of 0.0017 to 0.034 µg/L, 0.020 to >0.057 µg/L and 0.076 to >0.057 µg/L, respectively.

B.9.2.4.2 Reproductive and development toxicity to an additional aquatic invertebrate species

KIIA 8.3.2.1/04 (newly submitted with renewal dossier)

Author:	Schwader, A.L.
Title:	Beta-Cyfluthrin –Life Cycle Toxicity Test with Mysids (*Americamysis bahia*)
Date:	18 September 2013
Doc ID:	
Report no.:	EBFRL028
Edition no.:	13798.6307
Guidelines:	OCSPP Draft Guideline 850.1350
GLP:	yes
Validity:	valid

Deviations: None
At test termination, the mysids in the control and solvent control met the performance criteria of the OPPTS 850.1350 guideline (> 70 % survival of F0 mysids between pairing and exposure termination, >75 % of the females in the control and solvent control released young, and the control and solvent control organisms produced > 3 offspring per female). Post-pairing survival for the control and solvent control mean was 95 % and 89 %, respectively. Percentage of reproductively active females for both

control and solvent control mysids was 100 % for all replicates. The reproduction of mysids exposed to the control and solvent control ranged from 19.0 to 24.8 and 20.2 to 27.6 offspring per female, respectively. The control and solvent control mean was 21.5 and 23.8 offspring per female, respectively. No behavioral abnormalities were observed during the exposure period.

Materials:

Test item: Beta-cyfluthrin (BCS-AH45780)
Purity: 99.2 % w/w;
Batch: ABIDBBB085;
Test organism: Mysids *(Americamysis bahia),* ≤ 23 hours old.

Study Design and Methods:

Mysids were exposed in a chronic test for 28 days under flow-through conditions to five nominal test concentrations of 0.25, 0.50, 0.99, 2.0 and 4.0 ng/L (corresponding to mean measured concentrations of 0.11, 0.23, 0.41, 0.83 and 1.5 ng/L), a dilution water control and solvent control (acetone). Four replicates were maintained for each treatment and the controls. Each exposure aquarium contained one retention chamber, yielding 20 mysids per replicate vessel.
Survival of mysids (F0 generation) was estimated until day 12 (due the rapid movement of mysids in a single chamber containing up to 20 mysids) and counted thereafter daily. At day 12 mature mysids were paired. During the reproductive phase groups of 10 offspring F1 mysids per replicate (40 per treatment) were placed in a separate pairing chamber and monitored for 96 hours post-release for survival and behavior. For each replicate aquarium the total number of offspring produced per female was assessed. Furthermore the mean total body length and the dry weight of the parental generation were determined. Test conditions: 28-day duration, temperature range of 26 to 28 ºC, illumination of 16 hours light (270 to 390 lux) and 8 hours darkness. Diluted, filtered, natural seawater (salinity range of 19 to 21‰ and a pH range of 7.7 to 8.2) was used as dilution and control water.

Dates of experimental work: May 03, 2013 to May 31, 2013

Results and Discussion:

Analytical results
Mean measured concentrations of beta-cyfluthrin in the five test levels ranged from 37 % to 46 % of nominal concentrations. Based on mean measured beta-cyfluthrin concentrations, the treatment levels are defined as follows: 0.11, 0.23, 0.41, 0.83 and 1.5 ng/L.

Biological results

Table B.9.2-17 Summary of the first generation (F_0) survival at termination of the 28-day life-cycle exposure of mysids (*Americamysis bahia*) and of F_1 survival at 96-hours post release following of mysids (*Americamysis bahia*) to beta-cyfluthrin

Mean Measured concentration (ng/L)	Mean survival of F_0 male mysids [%]	Mean survival of F0 female mysids [%]	Mean survival F0 of mysids [%]	Mean survival among F1 mysids following 96 hours [%]
Control	90	100	81	100
Solvent control	86	94	78	100
Pooled control	88	97	80	100
0.11	74	98	78	95
0.23	88	91	81	100
0.41	71	88	68	100
0.83	85	94	80	98
1.5	81	86	59	95

* = significant difference compared to the pooled control (Fisher's Exact Test with Bonferroni-Holm's Adjustment)

Since no concentration tested resulted in ≥ 50 % mortality, the 7, 14, 21 and 28-day LC_{50} values were empirically estimated to be > 1.5 ng/L, the highest mean measured beta-cyfluthrin concentration tested.

Table B.9.2-18: Summary of average total body length and average dry body weight measurements of firstgeneration (F_0) male and female mysids measured at the termination of the 28-day life-cycle testexposing mysids (*Americamysis bahia*) to beta-cyfluthrin

Mean Measured concentration (ng/L)	Average total body length of male mysids [mm]	Average total body length of female mysids [mm]	Average dry body weight of male mysids [mg]	Average dry body weight of female mysids [mg]
Control	7.10	7.45	0.80	1.12
Solvent control	7.09	7.41	0.81	1.13
Pooled control	7.09	7.43	0.81	1.13
0.11	6.91	7.40	0.82	1.15
0.23	7.08	7.47	0.90	1.19
0.41	6.88	7.30	0.89	1.13
0.83	6.83	7.17*	0.77	1.03
1.5	6.69	7.14*	0.81	0.92

Table B.9.2-19: Summary of the first generation (F_0) reproductive success (offspring per female) at termination of the 28-day life-cycle exposure of mysids (*Americamysis bahia*)

Mean Measured concentration (ng/L)	**Mean number of offspring per female**
Control	21.5
Solvent control	23.8
Pooled control	22.6
0.11	21.1
0.23	23.4
0.41	21.5
0.83	15.4*
1.5	11.7*

* = significant difference compared to the pooled control (Fisher's Exact Test with Bonferroni-Holm's Adjustment)

Based on mean measured concentrations of beta-cyfluthrin, female body length and reproduction (the most sensitive indicators of toxicity), the No-Observed-Effect Concentration (NOEC) was determined to be 0.41 ng/L. The Lowest-Observed-Effect Concentration (LOEC) for mysids was determined to be 0.83 ng/L..

Conclusions:

The 7, 14, 21 and 28-day LC50 values were empirically estimated to be > 1.5 ng/L. The NOEC was determined to be 0.41 ng/L, based on effect on female body length and mean number of offspring per female at the LOEC (0.83 ng/L).

B.9.2.4.3 Sediment dwelling organisms

Development and emergence in *Chironomus* species

The current Annex I listing of beta-cyfluthrin was based on a study with a SC formulation (125 g/L) of beta-cyfluthrin and an EC formulation (50 g/L) of cyfluthrin (both water spiked). New studies with beta-cyfluthrin technical were performed, according to the spiked water and spiked sediment designs which replace the EU agreed endpoints based on formulation studies for the Renewal of Approval.

The relevant endpoints of the studies are summarised in Table B.9.2-20 and Table B.9.2-21.

Table B.9.2-20: Chronic toxicity of beta-cyfluthrin to *Chironomus riparius* (spiked water)

Species	Test design	EC50 (µg as/L)	NOEC (µg as/L)	Reference	Reliability
Beta-Cyfluthrin					
Chironomus riparius	28 d, static water sediment system	EC_5 = 0.32[1] (nom) EC_{15}= 0.36[1] (nom) EC_{50} = 0.45[1] (nom)	-	KIIA 8.5.2/01 HBF/CH 17 Heimbach, 1997 M-055336-01-1 R-19111	valid
Chironomus riparius	28 d, static water sediment system	EC_{10} = 1.3 (nom) EC_{15} > 1.6 (nom) EC_{20} > 1.6 (nom) EC_{50} > 1.6 (nom)	0.4 (nom)	KIIA 8.5.2/02 D58720 Kimmel, 2014c M-481015-01-1 R-30154	valid
FBC-acid					
Chironomus riparius	Toxicity is addressed by the study with the active substance.				
FPB-aldehyde					
Chironomus riparius	Toxicity is addressed by the study with the active substance.				
DCVA					
Chironomus riparius	DCVA is a main metabolite of beta-cyfluthrin. It is built in sediment up to 23.7 %. The maximum measured concentration is 100 days after application. No study addressing the toxicity of DCVA to sediment dwellers is available. Toxicity is adressed by alternative information replacing experimental studies according EFSA GD (2013).). (KIIA 8.2.5.4/04)				

Values in bold: Endpoints used for risk assessment
nom: nominal initial
[1] tested formulation was SC 125

Table B.9.2-21: Chronic toxicity of beta-cyfluthrin to Chironomus riparius (spiked sediment)

Species	Test design	EC_X (mg as/kg)	NOEC (mg as/kg)	Reference	Reliability
Beta-Cyfluthrin					
Chironomus riparius	28 d, static water sediment system spiked sediment	emergence rate: EC10 = 0.20 (nom) EC15 = 0.25 (nom) EC20 = 0.30 (nom) EC50 = 0.65 (nom) development rate: EC10 = 0.17 (nom) EC15 = 0.95(nom)	emergence rate: 0.125 (nom) development rate: < 0.125 (nom)	KIIA 8.5.2/03 D58731 Kimmel, 2014d M-481037-01-1 R-30153	valid

		EC20 = 0.30 (nom) EC50 > 2.0 (nom)			

nom: nominal initial

KIIA 8.5.2/01

Author:	Heimbach, F.
Title:	Influence of beta-Cyfluthrin SC 125 on development and emergence of larvae of *Chironomus riparius* in a water-sediment system
Date:	20 August 1997
Doc ID:	M-055336-01-1
Report no.:	HBF/CH 17
Guidelines:	BBA-guideline proposal: "Effects of plant protection products on the development of sediment dwelling larvae of *Chironomus riparius* in a water-sediment system." Mitteilung aus der Biol. Bundesanstalt, Heft 315, Blackwell Berlin, 1995, pp 70 - 84
GLP:	yes
Validity:	valid

Deviations: only three test concentrations used, whereas five are recommended according to current OECD Guideline No. 233

Test material: beta-Cyfluthrin SC 125, as content: 11.5 %, batch no. 0155 according to 03854/0145

Results: The EC_{15} (probit analysis) for numbers of emerged midges was 0.36 µg initial nominal as/L (confidence limits 0.22 - 0.60), the EC_5 0.32 µg as/L (confidence limits 0.19 - 0.52), the EC_{10} 0.34 µg as/L (confidence limits 0.21 - 0.55) and the EC_{50} 0.45 µg as/L (confidence limits 0.20 - 1.03).

Conclusion: EC_{50} = 0.45 µg as/L; EC_5 = 0.32 µg/L

KIIA 8.5.2/02 (newly submitted with the dossier)

Author:	Kimmel, S.
Title:	Beta-Cyfluthrin: Effects on the Development of Sediment-Dwelling Larvae of *Chironomus riparius* in a Water-Sediment System with Spiked Water
Date:	19 March 2014
Doc ID:	M-481015-01-1
Report no.:	D58720
Guidelines:	OECD Guideline No. 219: Sediment-Water Chironomid Toxicity Test Using Spiked Water (adopted 13 April 2004).
GLP:	yes
Validity:	valid

Deviations: None

Executive Summary

The purpose of this study was to evaluate effects of beta-cyfluthrin on the development of sediment dwelling larvae of the midge *Chironomus riparius* in water-sediment systems over 28 days. The test item was applied to the water column in static water-sediment systems. Twenty *Chironomus* larvae (4 collectives of 5 animals per test beaker) per concentration were exposed to 0.1, 0.2, 0.4, 0.8 and 1.6 µg as/L nominal concentrations. In addition, 20 *Chironomus* larvae were exposed to a test water control

(without test item) and a solvent control (80 µL DMF/L). Four replicates (test beakers) were tested in the biological test at each test concentration, in the control and the solvent control. The test parameters of the study were development time/rate of the midges and the emergence ratio (sum of fully emerged male and female midges divided by the number of larvae introduced into the system). *Chironomus riparius* were observed daily from day 10 to 28 and were fed at least three times per week during the test.
The analytically determined test item concentrations in the application solution samples after application corresponded to 87-92 % of the initial nominal test concentrations.
The mean measured concentrations of beta-cyfluthrin (sum of all isomers) in the water columns after the test item application on day 0 ranged from 25 to 27 % of the nominal concentrations in both analysed test concentrations of 0.4 and 1.6 µg as/L. The concentrations of beta-cyfluthrin in the water columns decreased rapidly during the test period, with recovered values down to 3 and 4 % on days 1 and 3, and 1 % on day 7 after test item application, respectively. At study termination (on day 28), all analytical measured concentrations were found to be below LOQ.
The concentrations found in the pore water samples increased from day 0 to 3 followed by a decrease. On day 0 mean measured concentrations were 0.0188 and 0.0959 µg as/L for the test concentrations of nominally 0.4 and 1.6 µg as/L, respectively. Throughout the following days, 1, 3 and 7, the mean measured concentration were 0.0479, 0.0771 and 0.0391 µg as/L for the nominal concentration of 0.4 µg as/L, and 0.123, 0.136 and 0.117 µg as/L for the nominal concentration of 1.6 µg as/L. At the end of the experiment at day 28 measurement at both concentrations were below LOQ.
In the sediment samples, the concentrations of beta-cyfluthrin (sum of all isomers) at all evaluated time points and for all analytically measured concentrations were <LOQ.
All reported biological results are related to the nominal initial concentrations of the test item in the water column.
All validity criteria according to the guideline OECD 219 were fulfilled.

The overall 28-day NOEC of beta-cyfluthrin for *Chironomus riparius* in this water-sediment study was determined to be 0.4 µg beta-cyfluthrin/L.
The EC_{10} was determined to be 1.3 µg as/L and the EC_{15}, EC_{20} and EC_{50} were all > 1.6 µg as/L.
The overall 28-day LOEC was determined to be at the nominal concentration of 0.8 µg beta-cyfluthrin/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Lot/Batch #:	PNBC000623
Purity:	99.3 % w/w
2. Vehicle and/or positive control:	N,N-Dimethylformamide (DMF)
3. Test organisms:	
Species:	*Chironomus riparius*
Age:	First instars (2-3 days)
Source:	Laboratory bred
Loading:	20 organisms per vessel (600 mL glass beakers)

4. Environmental conditions:

Temperature:	20.2 to 21.1 °C
Photoperiod:	Light/dark 16/8 h
Light intensity:	780 to 985 lux
pH:	8.1 to 8.6
Dissolved oxygen:	7.3 to 9.0 mg O_2/L (= at least 80 % oxygen saturation value)
Hardness:	200 mg/L $CaCO_3$

B. STUDY DESIGN

1. Experimental treatments

The effects of beta-cyfluthrin on the development of sediment-dwelling larvae of the midge *Chironomus riparius* in water-sediment systems were evaluated in a 28 days static toxicity test. Twenty larvae (4 collectives of 5 animals per test beaker) per concentration were exposed to 0.1, 0.2, 0.4, 0.8 and 1.6 µg as/L nominal concentrations. In addition, twenty larvae were exposed to a test water control (without test item) and a solvent control (80 µL DMF/L). Four replicates (test beakers) were tested in the biological test at each test concentration, in the control and the solvent control. First-instar larvae of *Chironomus riparius* were exposed to the test item for 28 days to assess the impact on full maturation of the larvae to adult midges. Twenty larvae of the first larval stage (2-3 days old) were allocated randomly to each test vessel. One day after adding the larvae, the test item was applied to the water column of the water-sediment systems (day 0). The test animals were fed at least three times per week.

2. Observations

The emergence and development of *Chironomus riparius* larvae (male and female midges) exposed to beta cyfluthrin were observed daily from day 10 to 28 .Water temperature, pH and concentration of dissolved oxygen were measured in all test vessels before insertion of the larvae. During the larval exposure period, these parameters were measured once per week and at study termination.
The water temperature was additionally measured twice per week. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from all application solutions immediately after the test item application. Further samples (water, pore water and sediment samples) were taken from the water-sediment system on day 0, 1, 3 and 7 for the determination of the test item concentration.

3. Statistical calculations

The mean emergence ratios and development rates of all test concentrations were statistically evaluated on significant differences to the solvent control by the multivariate Williams or Dunnett ttest for homogenous variances after a one-way analysis of variance (ANOVA). Statistical evaluations were done separately for emerged males and females (development rate) and with pooled sexes (emergence ratio). The mean emergence ratio and development rate of males and females in the control were compared to the solvent control by Student t-tests.
The 28-day EC_{10}, EC_{15}, EC_{20} and EC_{50} of the emergence ratio and the development rates could not be calculated since no toxic effect occurred up to and including the highest tested concentration, except the development rate for male midges, where the EC_{10} value was calculated by means of a Probit analysis using maximum linear likelihood regression.

II. RESULTS AND DISCUSSION

Findings:
Based on the nominal initial test item concentrations of beta-cyfluthrin, the following results for emergence and development rates after 28 days of exposure were assessed.

Results after 28 days	**Emergence rate (arcsin transformed) of pooled sexes (mg as/kg, nominal)**	**Development rate (µg/L, nominal)**	
		Males	**Females**
EC_{10}:	> 1.6	1.3	> 1.6
95 % confidence interval:	n.d.	0.93 - 2.0	n.d.
EC_{15}:	> 1.6	> 1.6	> 1.6
95 % confidence interval:	n.d.	n.d.	n.d.

EC_{20}: 95 % confidence interval:	> 1.6 n.d.	> 1.6 n.d.	> 1.6 n.d.
EC_{50}: 95 % confidence interval:	≥1.6 n.d.	> 1.6 n.d.	> 1.6 n.d.
NOEC:	≥1.6	0.4	0.8
LOEC:	> 1.6	0.8	1.6

n.d.: could not be determined due to minor effects of the test item on the development rate

During the test period, the pH values in the test media ranged from 8.1 to 8.6. The dissolved oxygen concentrations were at least 7.3 mg/L (= at least 80 % oxygen saturation value), and thus sufficiently high throughout the test period. The water temperature varied between 20.2 and 21.1 °C and was thus sufficiently constant. The water temperature differed by less than 1.0 °C between all beakers at any time during the study.
Analytical data: The analytically determined test item concentrations in the application solution Samples after application corresponded to 87-92 % of the initial nominal test concentrations. Therefore, all reported biological results are related to the nominal initial concentrations of the test item in the water column. The mean measured concentrations of beta-cyfluthrin (sum of all isomers) in the water columns after the test item application on day 0 ranged from 25 to 27 % of the nominal concentrations in both analysed test concentrations of 0.4 and 1.6 µg as/L. The concentrations of beta-cyfluthrin in the water columns decreased rapidly during the test period, with recovered values down to 3 and 4 % on days 1 and 3, and 1 % on day 7 after test item application, respectively. At study termination (on day 28), all analytical measured concentrations were found to be below LOQ.
The concentrations found in the pore water samples increased from day 0 to 3 followed by a decrease. On day 0 mean measured concentrations were 0.0188 and 0.0959 µg as/L for the lower and higher test concentrations of nominally 0.4 and 1.6 µg as/L, respectively. Throughout the following days, 1, 3 and 7, the mean measured concentration were 0.0479, 0.0771 and 0.0391 µg as/L for the lower nominal concentration of 0.4 µg as/L, and 0.123, 0.136 and 0.117 µg as/L for the higher nominal concentration of 1.6 µg as/L. At the end of the experiment at day 28 measurement at both concentrations were below LOQ.
In the sediment samples, the concentrations of beta-cyfluthrin (sum of all isomers) at all evaluated time points and for all analytically measured concentrations were found to be below the limit of quantification.

B. OBSERVATIONS

The emergence ratios per vessel in the control and solvent control ranged from 80 to 100 % (thus fulfilling the guideline validity criterion).
Up to and including the highest nominal test concentration of initial 1.6 µg/L, the mean emergence ratios of pooled sexes were not statistically significantly lower than in the control.

Table 9.2-22: Emergence ratio (Male and Female Midges Pooled)

	Control	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [µg/L]**				
			0.125	**0.25**	**0.5**	**1.0**	**2.0**
Sum of inserted larvae per treatment	80	80	80	80	80	80	80
Sum of emerged midges per treatment	78	73	67	74	68	61	69
% of emerged midges per treatment (mean)	97.5	91.3	83.8	92.5	85.0	76.3	86.3

Emergence ratio ERarc: Mean	1.490	1.320	1.170	1.300	1.220	1.080	1.240
SD	0.161	0.195	0.150	0.056	0.236	0.182	0.223
Min	1.250	1.110	1.050	1.250	1.050	0-940	1.110
Max	1.570	1.570	1.350	1.350	1.570	1.350	1.570
N	4	4	4	4	4	4	4
% of solvent control	112.9	100.0	88.6	98.5	92.4	81.8	93.9
STAT	n.s*	---	n.s	n.s	n.s	n.s	n.s

ERarc : arcsin-transformed emergence ratio
STAT : results of a Williams t-test (α = 0.05, one-sided smaller)
n.s: mean ERarc not statistically significantly lower than in the solvent control
n.s *: mean development rate not statistically significantly lower than in the solvent control (based on a Student t-test, α = 0.05, two-sided)

The midges in the control had emerged between days 12 and 21 (and thus fulfilled the validity criterion of the test guideline). Up to and including the tested concentration of initial 0.4 µg as/L, the mean development rates of males and females were not statistically significantly lower than in the control. From the nominal concentration of initially 0.8 µg as/L on, the mean development rate was slightly, but statistically significantly reduced.

Table B.9.2-23: Development Rate for Males and Females

Males							
Development rate per treatment (day-1)	**Solvent Control**	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [µg/L]**				
			0.125	**0.25**	**0.5**	**1.0**	**2.0**
Mean	0.070	0.070	0.070	0.070	0.070	0.070	0.060
SD	0.001	0.001	0.002	0.002	0.004	0.004	0.001
Min	0.070	0.070	0.070	0.070	0.070	0.060	0.060
Max	0.070	0.070	0.070	0.070	0.070	0.070	0.060
N	4	4	4	4	4	4	4
% of solvent control	100.00	100.0	100.0	100.0	100.0	100.0	85.7
STAT	n.s*	---	n.s	n.s	n.s	s.	s.
Females							
Development rate per treatment (day-1)	**Solvent Control**	**Control**	**Beta-Cyfluthrin, nominal concentration [mg/kg dry sediment]**				
			0.125	**0.25**	**0.5**	**1.0**	**2.0**
Mean	0.060	0.060	0.060	0.060	0.060	0.060	0.060
SD	0.003	0.003	0.006	0.001	0.002	0.001	0.004
Min	0.060	0.060	0.050	0.060	0.060	0.060	0.050
Max	0.060	0.070	0.070	0.060	0.060	0.060	0.060
N	4	4	4	4	4	4	4
% of solvent control	100.0	100.0	100.0	100.0	100.0	100.0	100.0
STAT	n.s*	---	n.s	n.s	n.s	n.s	s.

STAT : results of a Williams t-test (males) or Dunnett t-test (females, α = 0.05, one-sided smaller)
n.s: mean development rate not statistically significantly lower than in the solvent control
s.: mean development rate statistically significantly lower than in the solvent control
n.s *: mean development rate not statistically significantly lower than in the solvent control (based on a Student t-test, α = 0.05, two-sided)

No symptoms of toxicity were observed at the larvae, pupae and emerged midges during the study.

III. CONCLUSIONS

The overall 28-day NOEC of beta-cyfluthrin for Chironomus riparius in this water-sediment study was determined to be 0.4 µg beta-cyfluthrin/L. The EC_{10} was determined to be 1.3 µg as/L and the EC_{15}, EC_{20} and EC_{50} were all > 1.6 µg as/L.
The overall 28-day LOEC was determined to be at the nominal concentration of 0.8 µg beta-cyfluthrin/ L.

KIIA 8.2.5.4/03 (newly submitted with the dossier)

Author:	Kimmel, S.
Title:	Beta-Cyfluthrin: Effects on the Development of Sediment-Dwelling Larvae of *Chironomus riparius* in Water-Sediment Systems with Spiked Sediment
Date:	21 March 2014
Doc ID:	M-481037-01-1
Report no.:	D58731
Guidelines:	OECD Guideline No. 218: Sediment-Water Chironomid Toxicity Test Using Spiked Sediment (adopted 13 April 2004).
GLP:	yes
Validity:	valid

Deviations: None
Dates of experimental work: 02 November 2012 to 27 August 2013
Executive Summary
The purpose of this study was to evaluate effects of the test item beta-cyfluthrin on the development ofsediment-dwelling larvae of the midge *Chironomus riparius* in water-sediment systems over 28 days.
Twenty *Chironomus larvae* (4 collectives of 5 animals per test beaker) per concentration were exposed to 0.125, 0.25, 0.5, 1.0 and 2.0 mg as/kg dry sediment nominal concentrations. In addition, 20 *Chironomus* larvae were exposed to a test water control (without test item) and a solvent control (1 mL chloroform/10g sand). Four replicates (test beakers) were tested in the biological test at each test concentration, in the control and the solvent control. The endpoints of the study were the emergence ratio (sum of fully emerged midges divided by the number of inserted larvae) and the development time/rate of the test animals. *Chironomus riparius* were observed daily from day 10 to 28 and were fed at least three times per week during the test. Further samples (water, pore water and sediment samples) were taken from the water-sediment system on day -2, -1, 0, 7 and 28 from the water-sediment systems (nominal 0.125 and 2.0 mg as/kg dry sediment) for the determination of the test item concentrationst.
The analytically measured concentrations of beta-cyfluthrin in the application solution samples ranged from 94 to 100 % of the initial nominal values.
The mean measured concentrations in the water columns after the test item application on day- 2 were 0.0249 and 0.221 µg as/L in both analysed test concentrations of 0.125 and 2.0 mg as/kg dry sediment, respectively. Concentrations in the water columns decreased in the water-sediment system during the test period. At day -1 they were 0.00463 and 0.0472 µg as/L, and on day 0 at 0.00406 and 0.0660 µg as/L. Seven days after the test item application, the concentrations in the water columns were 0.00784 and 0.0339 µg as/L, and at test end at day 28, the concentrations was below the LOQs of 0.001 and 0.0341 µg as/L, respectively.
In pore water, the concentrations of beta-cyfluthrin fluctuated during the study period in both analysed test concentrations. After test item application at day -2 in both analysed test concentrations of 0.125 and 2.0 mg as/kg dry sediment, concentrations in pore water were 0.289 and 4.97 µg as/L, whereas at day -1 1.05 and 2.33 µg as/L were measured. Measurements resulted in 0.798 and 25.5 µg as/L at day 0, in 0.615 and 8.63 µg as/L seven days after the test item application. At test end at Day 28, pore water concentrations were 0.230 and 5.74 µg as/L.
In the sediment samples, the concentrations of beta-cyfluthrin (sum of all isomers) remained more or less stable during the study period. At both nominal test item concentrations of 0.125 and 2.0 mg as/kg

dry sediment the measured concentrations corresponded to 91-95 % of nominal at day -2, to 92-100 % at day -1, to 96-98 % at day 0, to 88-91 % at day 7 and to 90-110 % of nominal at test end.

All reported biological results are related to the nominal initial concentrations of the test item. This is also supported by the analytical results of the sediment samples of both measured concentrations, which remained stable between 88-110 % of nominal throughout the whole test period.
All validity criteria according to the guideline OECD 218 were fulfilled.

In regard to the emergence rate, the following the following values were determined:
28-day EC_{10} = 0.20 mg as/kg dry sediment
(confidence limits: 0.038 -0.34 mg as/kg dry sediment)
28-day EC_{20} = 0.25 mg as/kg dry sediment
(confidence limits: 0.062 -0.41 mg as/kg dry sediment)
28-day EC_{50} = 0.65 mg as/kg dry sediment
(confidence limits: 0.38 - 1.2 mg as/kg dry sediment)

In regard to the development rate, the following the following values were determined:
28-day EC_{10} = 0.17 mg as/kg dry sediment
(confidence limits: 0.07 -0.27 mg as/kg dry sediment)
28-day EC_{20} = 0.95 mg as/kg dry sediment
(confidence limits: 0.69 -1.4 mg as/kg dry sediment)
28-day EC_{50} > 2.0 mg as/kg dry sediment
(confidence limits: n.d.)

The EC_{15} was 0.25 mg as/kg, the EC_{20} was 0.30 mg as/kg and the EC_{50} was 0.65 mg as/kg. The overall 28-day LOEC was determined to be at ≤0.125 mg as/kg dry sediment due to a statistically significantly reduced development rate of the midges.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin technical
Lot/Batch #:	PNBC000623
Purity:	99.3 % w/w
2. Vehicle and/or positive control:	Chloroform
3. Test organisms:	
Species:	*Chironomus riparius*
Age:	First instars (2-3 days)
Source:	Laboratory bred
Loading:	20 organisms per vessel (600 mL glass beakers)
4. Environmental conditions:	
Temperature:	20.1 to 21.1 C°
Photoperiod:	Light/dark 16/8 h
Light intensity:	590 to 960 lux
pH:	8.0 to 8.6
Dissolved oxygen:	7.2 to 8.6 mg O2/L (= at least 85 % oxygen saturation value)
Hardness:	3.4-3.6 mmol/L

B. STUDY DESIGN

1. Experimental treatments
The effects of beta-cyfluthrin on the development of sediment-dwelling larvae of the midge *Chironomus riparius* in water-sediment systems were evaluated in a 28 days static toxicity test. Twenty larvae (4 collectives of 5 animals per test beaker) per concentration were exposed to 0.125, 0.25, 0.5, 1.0 and 2.0 mg as/kg dry sediment nominal concentrations. In addition, twenty larvae were exposed to a test water control (without test item) and a solvent control. Four replicates (test beakers)

were tested in the biological test at each test concentration, the control and the solvent control. First-instar larvae of *Chironomus riparius* were exposed to the test item for 28 days toassess the impact on full maturation of the larvae to adult midges.
Twenty larvae of the first larval stage (2-3 days old) were allocated randomly to each test vessel. The larvae were inserted into the test vessels two days after spiking the sediment and establishing the water-sediment systems. The day of the larvae insertion was defined as Day 0 of the study. The test animals were fed at least three times per week.

2. Observations

The emergence and development of *Chironomus riparius* larvae (male and female midges) exposed to beta cyfluthrin were observed daily from day 10 to 28 .Water temperature, pH and concentration of dissolved oxygen were measured in all test vessels before insertion of the larvae. During the larval exposure period, these parameters were measured once per week and at study termination.
The water temperature was additionally measured twice per week. Samples for the determination of the concentrations of beta-cyfluthrin in the test medium were taken from all application solutions immediately after the test item application. Further samples (water, pore water and sediment samples) were taken from the water-sediment system on day -2, -1, 0, 7 and 28 for the determination of the test item concentration.

3. Statistical calculations

For both parameters, emergence ratio and development rate the arithmetic mean values (mean), deviation (SD), minimum and maximum (min/max) were calculated from the four replicates per treatment.
The mean emergence ratios and development rates of all test concentrations were statistically evaluated on significant differences to the control by the multivariate Dunnett t-test after a one-way analysis of variance (ANOVA). Statistical evaluations were done separately for emerged males and females (development rate) and with pooled sexes(emergence ratio).
The 28-day EC10, EC15 and EC20 of the emergence ratio and development rate and the EC50 of the emergence ratio were calculated by means of a Probit analysis using linear maximum likelihood regression.
The 28-day EC50 of the development rate could not be calculated since none of the responses in the test item treatment exceeded 50 %.

II. RESULTS AND DISCUSSION

Findings:
Based on the nominal initial test item concentrations of beta-cyfluthrin, the following results for emergence and development rates after 28 days of exposure were assessed.

Results after 28 days	**Emergence rate (arcsin transformed) of pooled sexes (mg as/kg, nominal)**	**Development rate (mg as/kg, nominal)**		
		Males	**Females**	**Males and females**
EC_{10}: 95 % confidence interval:	0.20 0.038-0.34	0.20 0.082-0.31	0.34 0.13-0.54	0.17 0.07-0.27
EC_{15}: 95 % confidence interval:	0.25 0.062-0.41	0.43 0.26-0.60	0.81 0.51-1.4	0.44 0.28-0.61
EC_{20}: 95 % confidence interval:	0.30 0.092-0.48	0.80 0.57-1.1	1.6 1.0- > 2.0	0.95 0.69-1.4

EC_{50}: 95 % confidence interval:	0.65 0.38-1.2	> 2.0 n.d.	> 2.0 n.d.	> 2.0 n.d.
NOEC:	0.125	< 0.125	< 0.125	< 0.125
LOEC:	0.25	≤ 0.125	≤ 0.125	≤ 0.125

n.d.: could not be determined due to minor effects of the test item on the development rate

During the test period, the pH values in the test media ranged from 8.0 to 8.6. The dissolved oxygen concentrations were at least 7.2 mg/L (= 85 % oxygen saturation value), and thus, sufficiently high throughout the test period. The water temperature varied between 20.1 and 21.1 °C and was thus sufficiently constant. The water temperature differed by less than 1.0 °C between all beakers at any time during the study.

Analytical data

The analytically determined test item concentrations in the application solution samples after application ranged from 94 to 100 % of the initial nominal test concentrations. Therefore, all reported biological results are related to the nominal initial concentrations of the test item.

The mean measured concentrations of beta-cyfluthrin (sum of all isomers) in the water columns after the test item application on day- 2 were determined to be 0.0249 and 0.221 µg as/L in both analysed test concentrations of 0.125 and 2.0 mg as/kg dry sediment.. The concentrations of beta-cyfluthrin in the water columns decreased in the water-sediment system during the test period. At day -1, concentrations in the water phase were at 0.00463 and 0.0472 µg as/L, while on day 0 at 0.00406 and 0.0660 µg as/L.

Seven days after the test item application, the concentrations in the water columns were determined to be at 0.00784 and 0.0339 µg as/L, and at test end at day 28, the concentrations in the water phase were determined to be below the limit of quantification (LOQ = 0.001 µg test item/L) and 0.0341 µg as/L.

In the pore water, the concentrations of beta-cyfluthrin fluctuated during the study period in both analysed test concentrations. After test item application at day -2 in both analysed test concentrations of 0.125 and 2.0 mg as/kg dry sediment, concentrations in the pore water were determined to be 0.289 and 4.97 µg as/L, whereas at day -1 concentrations were determined to be 1.05 and 2.33 µg as/L. At day 0, concentrations in the pore water were at 0.798 and 25.5 µg as/L, seven days after the test item application concentrations in the pore water were determined to be 0.615 and 8.63 µg as/L. At test end at Day 28, the concentrations in the pore water were determined to be 0.230 and 5.74 µg as/L.

In the sediment samples, the concentrations of beta-cyfluthrin (sum of all isomers) remained more or less stable during the study period. At both nominal test item concentrations of 0.125 and 2.0 mg as/kg dry sediment the measured concentrations corresponded to 91-95 % of nominal at day -2, to 92-100 % at day -1, to 96-98 % at day 0, to 88-91 % at day 7 and to 90-110 % of nominal at test end.

B. OBSERVATIONS

The emergence ratios per vessel in the control and solvent control ranged from 70 to 95 % (thus fulfilling the guideline validity criterion).

From the second lowest tested nominal concentration of initial 0.25 mg/kg dry sediment on, the mean emergence ratios of pooled sexes was statistically significantly lower than in the control and solvent.

Table B.9.2-24: Emergency ratio (Male and Female Midges Pooled)

	Control	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [mg/kg dry sediment]**				
			0.125	**0.25**	**0.5**	**1.0**	**2.0**
Sum of inserted larvae per treatment	80	80	80	80	80	80	80
Sum of emerged midges per treatment	73	69	67	57	55	17	8.0

% of emerged midges per treatment (mean)	91.3	86.3	83.9	71.3	68.8	21.3	10.0
Emergence ratio ERarc: Mean	1.280	1.210	1.170	1.020	0.980	0.470	0.270
SD	0.0830	0.1520	0.1290	0.1770	0.0520	0.1110	0.2070
Min	1.170	0.990	1.050	0.840	0.940	0.320	0.000
Max	1.350	1.350	1.350	1.250	1.050	0.580	0.460
N	4	4	4	4	4	4	4
% of solvent control	105.8	100.0	96.7	84.3	81.0	38.8	22.3
STAT	n.s*	---	n.s-	s.	s.	s.	s.

ERarc : arcsin-transformed emergence ratio
STAT : results of a Williams t-test (α = 0.05, one-sided smaller)
n.s: mean ERarc not statistically significantly lower than in the pooled controls
s.: mean ERarc statistically significantly lower than in the pooled controls
n.s *: mean development rate not statistically significantly different from the solvent control (based on a Student t-test, α = 0.05, two-sided)
Note: Since no statistically significantly effect between control and solvent control was detected, both controls were pooled for statistical evaluation of the test item concentrations.

The midges in the control had emerged between days 13 and 23 (and thus fulfilled the validity criterion of the test guideline), whereas two midges in the solvent control (one each in replicate 1 and 2) emerged on day 25. Since these two individuals do not have a statistically significant influence on the results, this deviation to the guideline criterion is not seen as biologically relevant and does not affect the outcome of the study.
From the lowest tested nominal concentration of initial 0.125 mg as/kg dry sediment on, the mean development rate of both sexes was statistically significantly lower than in the control and solvent control.

Table B.9.2-25: Development Rate for Males and Females

Males							
Development rate per treatment (day-1)	**Control**	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [mg/kg dry sediment]**				
			0.125	**0.25**	**0.5**	**1.0**	**2.0**
Mean	0.067	0.069	0.062	0.061	0.059	0.052	0.049
SD	0.0009	0.0028	0.0031	0.0037	0.0043	0.0044	0.0011
Min	0.066	0.066	0.058	0.055	0.052	0.046	0.048
Max	0.068	0.072	0.065	0.063	0.061	0.056	0.050
N	4	4	4	4	4	4	3
% of solvent control	97.1	100.0	89.9	88.4	85.5	75.4	71.0
STAT	n.s*	---	n.s	s.	s.	s.	s.
Females							
Development rate per treatment (day-1)	**Control**	**Solvent Control**	**Beta-Cyfluthrin, nominal concentration [mg/kg dry sediment]**				
			0.125	**0.25**	**0.5**	**1.0**	**2.0**
Mean	0.058	0.056	0.053	0.061	0.059	0.052	0.049
SD	0.0019	0.0023	0.0020	0.0037	0.0043	0.0044	0.0011
Min	0.056	0.054	0.051	0.055	0.052	0.046	0.048
Max	0.060	0.059	0.056	0.063	0.061	0.056	0.050
N	4	4	4	4	4	4	3
% of solvent control	103.6	100.0	94.6	94.6	87.5	91.1	76.8
STAT	n.s*	---	n.s	s.	s.	s.	s.

STAT : results of a Williams t-test (males) or Dunnett t-test (α = 0.05, one-sided smaller)

s.: mean development rate statistically significantly lower than in the solvent control
n.s *: mean development rate not statistically significantly lower than in the solvent control (based on a Student t-test, $\alpha = 0.05$, two-sided)
Note: Since no statistically significantly effect between control and solvent control was detected, both controls were pooled for statistical evaluation of the test item concentrations.

No symptoms of toxicity were observed at the larvae, pupae and emerged midges during the study.

III. CONCLUSIONS

The overall 28-day NOEC of beta-cyfluthrin for *Chironomus riparius* in this water-sediment study was determined to be <0.125 mg as/kg dry sediment.
In regard to the emergence rate, the following the following values were determined:
28-day EC_{10} = 0.20 mg as/kg dry sediment
(confidence limits: 0.038 -0.34 mg as/kg dry sediment)
28-day EC_{20} = 0.25 mg as/kg dry sediment
(confidence limits: 0.062 -0.41 mg as/kg dry sediment)
28-day EC_{50} = 0.65 mg as/kg dry sediment
(confidence limits: 0.38 - 1.2 mg as/kg dry sediment)

In regard to the development rate, the following the following values were determined:
28-day EC_{10} = 0.17 mg as/kg dry sediment
(confidence limits: 0.07 -0.27 mg as/kg dry sediment)
28-day EC_{20} = 0.95 mg as/kg dry sediment
(confidence limits: 0.69 -1.4 mg as/kg dry sediment)
28-day EC_{50} > 2.0 mg as/kg dry sediment
(confidence limits: n.d.)

The overall 28-day LOEC was determined to be at ≤ 0.125 mg as/kg dry sediment due to a statistically significantly reduced development rate of the midges.

KIIA 8.2.5.4/04 (newly submitted with the dossier)

Author:	Ducrot, V.
Title:	Aquatic ecotoxicology: Supporting information for the risk assessment of the beta-cyfluthrin metabolite DCVA to sediment dwellers.
Date:	11 November 2015
Doc ID:	M-538891-01-1
Report no.:	-
Guidelines:	-
GLP:	-
Validity:	-

The EFSA AGD (2013) makes recommendation for testing, non-testing and risk assessment of major aquatic metabolites from an active substance (point 10.2.4, page 143 of the EFSA AGD). These recommendations were followed in order to assess whether or not a new chronic laboratory study with *C. riparius* and the metabolite DCVA is required as support to the risk assessment of this metabolite. The EFSA GD (2013) states that a sediment study is "obligatory only if the substance partitions to sediment and/or when the substance interferes with moulting hormones (e.g. insect growth regulators)". The substance is considered to partition to the sediment if the water/sediment study shows >10 % of applied radioactivity at/or after 14 days present in the sediment and the chronic Daphnia test (or other comparable study with e.g. *Chironomus*) shows a EC_{10}/NOEC of < 0.1 mg/L". Additionally, the EFSA AGD (2013) states "Clearly, the potential to exclude testing on the basis of toxicity will depend on the data available for the metabolite. The applicant should therefore make a case as to whether sediment testing can be waived based on what is known about the fate properties and toxicity profile of

the metabolite. For example, if RAs with *Daphnia* indicate that the potential risks are low (taking into account the exposure situation in the sediment), then no further testing should in general be required". The need for a sediment test with DCVA was evaluated below, based on these criteria.

Partition to the sediment and toxicity of DCVA to *Daphnia magna*

DCVA reached a maximum of 23.6 % of the applied radioactivity in the sediment in the water sediment study conducted with cyclopropyl-labelled cyfluthrin (Sneikus 2000, M-022319-02-1). Based upon this environmental fate study, partition of DCVA to sediment is > 10 %; chronic risk of DCVA to sediment dwellers should thus be addressed. Either testing or non-testing approaches can be used in order to do so (EFSA GD 2013).

Available toxicity data for daphnid and DCVA raise no concern to aquatic invertebrates. In an acute test with *Daphnia magna* an EC_{50} of 25 mg/L DCVA was obtained (Forbis and Burgess 1984, M-034747-01-1). Additional information is available from a publication (Hill 1989, M-090574-01-1) that provided an EC_{50} of 130 mg/L DCVA. Based upon these results, DCVA is by far less toxic (i.e. at five orders of magnitude) than the parent compound beta-cyfluthrin (for which an EC_{50} of 0.000105 mg as/L was found in daphnids; Kimmel 2014a, M-481046-01-1). Based on the low acute toxicity of DCVA to daphnids, chronic studies with daphnid and DCVA are not required and thus not available. Acute studies with fish also support the finding that DCVA (LC_{50} = 14700 µg/L) is several order of magnitude less toxic than its parent beta-cyfluthrin (LC_{50} = 0.068 µg as/L).
As a conclusion, available ecotoxicological information shows that DCVA is far less toxic than beta-cyfluthrin to daphnids and fish. Based upon these results (and especially based on daphnid results), it is expected that DCVA is also far less toxic to *C. riparius* than beta-cyfluthrin.

Physico-chemical properties, structural properties analysis and efficacy data

Structural properties analysis and efficacy data are also available. They support the assumption that DCVA is not expected to be toxic to sediment dwellers. Indeed, the toxophore of beta-cyfluthrin is the intact ester function of the pyrethroid (Clark and Symington, 2011). The ester function needs to be intact to elicit toxicity. In the first degradation step in sediment water systems under aerobic conditions, there is a cleavage of the ester function leading to DCVA. Due to the loss of the ester function in DCVA, the toxophore of the molecule is lost: therefore, DCVA is not expected to have insecticidal activity.
This theoretical assumption based on the structural properties of DCVA is confirmed by the results from efficacy screening experiments in six species of insects or mites (Franken 2006, M-266539-01-1). Results from these experiment clearly show that DCVA has no remaining insecticidal or acaricidal activity even at the highest test concentration of 1000 ppm, while significant toxicity of beta-cyfluthrin and cyfluthrin was observed at low test concentrations 0.01 ppm.
Moreover, the water solubility of DCVA at 20 % and at pH 7.0 is 42 g/L (KCA 2.14 /13, Wiche and Ziemer, 2012, M-438162-01-1), while the water solubility of beta-cyfluthrin at 20 °C and pH 6.4 is 2.1 µg/L (KCA 2.5 /01, Sonnenschein 2013a, M-470002-01-1) and 1.6 µg/L (KCA 2.5 /02, Sonnenschein 2013b, M-470008-01-1) for isomer II and isomer IV, respectively. Log Po/w of DCVA at pH 7 and 25 °C is 0.8 (KCA 2.7 /05, Ziemer and Charter 2012, M-438168-01-1), while the Log Po/w of beta-cyfluthrin at 25 °C and pH 5.6 is 5.9 (KCA 2.7/01, Wiche et al. 2013a, M-447653-01-1) and 5.8 (KCA 2.7/02, Wiche et al. 2013b, M-447649-01-1) for isomer II and isomer IV, respectively. These data show that DCVA is much more hydrophilic than beta-cyfluthrin.
Finally, as shown by the results from fish, daphnid and algae tests, DCVA is several orders of magnitude less toxic than its parent compound beta-cyfluthrin.

Based on the above-mentioned information, DCVA is expected to have a lower risk to sediment dwellers than its parent beta-cyfluthrin.
For metabolites with such properties, the EFSA AGD (2013) as well as Sinclair and Boxall (2009) indicate that the toxicity of the metabolite is in general covered by that of the parent compound. Therefore, and as a conservative approach in line with EFSA AGD (2013) risk assessment scheme for me-

tabolites, tests conducted with beta-cyfluthrin and sediment dwellers can be used in order to address the risk of DCVA to sediment dwellers.

Two valid laboratory studies addressing the toxicity of beta-cyfluthrin to sediment dwellers are available: a spiked water-test with *C. riparius* (Kimmel 2014c, M-481015-01-1) and a spiked sediment-test with *C. riparius* (Kimmel 2014d, M-481037-01-1).
Due to the reasoning above, it can be assumed that the toxicity of DCVA is covered by the studies with the parent compound.

B.9.2.5 Effects on algal growth

Reference is made to the studies on which the current Annex I listing of beta-cyfluthrin is based (Heimbach, 1987 for beta-cyfluthrin; Hill, 1989 for the main metabolites).
The relevant endpoints of the studies are summarised inTable B.9.2-26.

The metabolites FPB-acid and DCVA of beta-cyfluthrin were considered to be of no environmental concern. In the Monograph it is stated “any harmful effects caused by these metabolites can obviously be precluded because of the data on toxicity” (Monograph, section B.7.9; Addendum 2 to Monograph, section 7.6).

Table B.9.2-26: Toxicity of beta-cyfluthrin and its metabolites to algea

Species	**Test design**	**EC_{50} (µg as/L)**	**Reference**	**reliability**
Beta-Cyfluthrin				
Scenedesmus subspicatus	96 h static	**E_rC_{50} > 2 E_bC_{50} > 2 (limit of solubility)** [ErC_{50} > 10 (nom) EbC_{50} >10 (nom)]	KIIA 8.4/01 HBF/AL 40 Heimbach, 1987 M-056512-01-1 R-19109	valid
FPB-acid				
Algae	-	EC_{50} >10000 (nom)	KIIA 8.4/02 Lit. 6002 Hill, 1989 M-090574-01-1 R-19095	Additional information
DCVA				
Algae	-	EC_{50} >10000 (nom)	KIIA 8.4/02 Lit. 6002 Hill, 1989 M-090574-01-1 R-19095	Additional information

nom: nominal initial

KIIA 8.4/01

Author:	Heimbach, F.
Title:	Growth inhibition of green algae (*Scenedesmus subspicatus*) caused by FCR 4545 (techn.)
Date:	27 August 1987
Doc ID:	M-056512-01-1

Report no.:	HBF/AL 40
Guidelines:	ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test) from 19.06.84 resp. OECD Guideline No. 201 "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (07.06.84).
GLP:	yes
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 201
Test material: beta-Cyfluthrin techn. (FCR 4545), purity: 98.0 %, batch no. 16001/87
Results: Beta-cyfluthrin technical (FCR 4545) was tested at one concentration of 0.01 mg as/L. Higher test concentrations could not be examined due to the low water solubility. No effects were seen at this concentration (NOEC ≥ 10 µg as/L for biomass and the growth rate). Accordingly the EC_{50} is > 10 µg as/L based on nominal test concentrations.

Conclusion: NOEC ≥ 10 µg as/L; EC_{50} > 10 µg as/L
However, the solubility limit of beta-cyfluthrin is 2 µg/L. Thus, the NOEC as well as the EC_{50} is > 2 µg/L.

KIIA 8.4/02

Author:	Hill, I. R
Title:	Aquatic organisms and pyrethroids
Date:	1990
Doc ID:	
Report no.:	Lit. 6002
Edition no.:	M-090574-01-1 (R-19095)
Guidelines:	Publisher: Society of Chemical Industry, Great Britain, Journal: Pesticide Science, Volume:27, Pages:429-457,
GLP:	No, publication
Validity:	supplementary data

Results: The FPB-acid EC_{50} for algae is reported to be >10000 µg/L. The DCVA EC50 for algae is reported to be >10000 µg/L.
The DCVA EC_{50} for algae is reported to be >10000 µg/L. The DCVA EC_{50} for algae is reported to be >10000 µg/L.

Conclusion: supplementary data

B.9.2.6 Effects on aquatic macrophytes

As beta-cyfluthrin is an insecticide, studies on the effects on aquatic plants are actually not required. However, a study with cyfluthrin and *Lemna gibba* was conducted for the US registration and is listed below for the sake of completeness (see Table B.9.2-27). The results of this study indicate low toxicity of the compound to aquatic plants, i.e. at the highest concentration tested (0.84 µg as/L, functional limit of solubility) maximum growth rate inhibitions of 5.3 % were found (dry weight of fronds).

Table B.9.2-27: Toxicity cyfluthrin to aquatic macrophytes

Species	Test design	EC50 (µg as/L)	Reference	reliability
Cyfluthrin				
Lemna gibba	7 d static-renewal	ErC50 > 0.84 (mean measured)	KIIA 8.6 M-437708-02-1 Banman et al. 2012	valid

B.9.2.7 Further testing on aquatic organisms

No further testing on aquatic organisms was performed with the active substance.

B.9.3 Effects on arthropods

B.9.3.1 Effects on bees (KCA 8.3.1)

There is an extensive regulatory database assessing the toxicity of beta-cyfluthrin to bees. Based on the available studies, the EU evaluation of beta-cyfluthrin (2002) concluded that there is a high risk from beta-cyfluthrin to bees, which is also confirmed by new data (refer to CP B.9.5.1.). Reference is made to those studies used in the EU review of beta-cyfluthrin (2002), which are also appropriate for the approval of renewal.

A summary of the data reviewed in the EU evaluation of beta-cyfluthrin (2002) are summarised in Table B.9.3-1.

Table B.9.3-1: Acute toxicity of beta-cyfluthrin and cyfluthrin to bees

Test substance	Test species	Endpoint	Value	Reference
beta-cyfluthrin tech.	honeybee	48 h acute oral LD_{50}	0.05 µg as/bee	Kleiner, 1996 SANCO/6841/VI/97-final
		48 h acute contact LD_{50}	0.012 µg as/bee	
cyfluthrin tech.	honeybee	24 h acute oral LD_{50}	0.051 µg as/bee	Davies *et al.*, 1985 SANCO/6841/VI/97-final
		24 h acute contact LD_{50}	0.0098 µg as/bee [1]	

[1] reported value of 0.001 µg/bee in the review report is considered a typographical error.

B.9.3.1.1 Acute toxicity (KCA 8.3.1.1.)

Acute oral (KCA 8.3.1.1.1) and contact (KCA 8.3.1.1.2) toxicity

Report: B 9.3.1.1/1
Davies, L. G. *et al*., 1985
Report on a laboratory investigation into the toxicity of cyfluthrin (Baythroid) to honey bees (*Apis melifera*)
Department of Life Science, Nottingham, UK; Report no.: TOX 1368

Guidelines: Pesticide safety Precaution Scheme, Working Document D3. Laboratory Tests for Toxicity to Honey bees
GLP: no

Executive Summary

The toxicity of cyfluthrin to honeybees was determined in laboratory tests. In contact tests the LD_{50} of cyfluthrin to honeybees was 0.0098 and in feed tests the LD_{50} was 0.051 µg/bee.

RMS's comments:

This study is considered valid and in principle acceptable for the risk assessment. However, the study is considered old and as newer, valid GLP-data are available (Kleiner *et al.*, 1996; reported in B 9.3.1.1/2) and the studies show only marginal differences of oral and contact toxicity, the study from Davies *et al.* is not used for risk assessment.

Report: B 9.3.1.1/2
Kleiner, R., 1996
Testing toxicity to honeybee - *Apis mellifera* L. (laboratory) according to EPPO Guideline No. 170 (1992) - FCR 4545 BioChem GmbH Karlsruhe, Cunnersdorf, Germany; Report no.: 96 10 48 079
Guidelines: OECD Guideline No. 214 (1992), OECD 214 (1992)
GLP: yes

Executive Summary

The study was performed according to EPPO Guideline No. 170 (1992). Two experiments, each with a duration of 48 hours, were conducted to determine the LD_{50} of the test substance FCR 4545 (beta-cyfluthrin) to the honeybee *Apis mellifera* L. in the oral toxicity test and in the contact toxicity test. Test criteria were mortality and behaviour of the honeybees in comparison with the control.

In the oral toxicity test as well as in the contact toxicity test exposure to FCR 4545 caused equivalent effects on honeybee mortality as the reference substance Dimethoate EC 400.

Test substance	Test species	Endpoint	Value (µg as/bee)
FCR 4545 (beta-cyfluthrin)	24 h	oral LD_{50}	0.05
		contact LD_{50}	0.021
	48 h	**oral LD_{50}**	**0.05**
		contact LD_{50}	**0.012**
Dimethoate EC 400	24 h	oral LD_{50}	0.31
		contact LD_{50}	0.33
	48 h	oral LD_{50}	0.28
		contact LD_{50}	0.26

In the test variant affected bees showed restlessness, irritation, uncontrollable motions and dorsal position before dying. 24 h after application surviving bees showed still apathetic or uncontrollable motions as well as dorsal position in some cases, whereas 48 h after application no behavioural anomalies were observed.

In the reference variant apathy, uncontrollable motions and dorsal position of affected bees could be observed before dying. 24 h and 48 h after application the surviving bees exhibited no behavioural anomalies.

Results: The LD_{50} of beta-cyfluthrin was 0.05 µg/bee in the oral toxicity test and 0.012 µg/bee in the contact toxicity test after 48 hours.

RMS's comments:
This study is considered valid and acceptable for the risk assessment.

B.9.3.1.2 Chronic toxicity (KCA 8.3.1.2)

No tests regarding the chronic toxicity of technical beta-cyfluthrin were submitted. However, a chronic toxicity of Bulldock 25 EC (25 g/L beta- cyfluthrin), to adult honey bees was assessed in a 10-day chronic feeding test (please refer to Sandrock C., 2014a, CP 9.5.1.2/1).

B.9.3.1.3 Effects on honeybee brood (KCA 8.3.1.3)

No tests regarding the effects of technical beta-cyfluthrin on honeybee brood were submitted. However, the acute toxicity of Bulldock 25 EC (25 g/L beta- cyfluthrin), to bee larvae was assessed in a laboratory study (please refer to Sandrock C., 2014b, CP 9.5.1.3/1).

B.9.3.1.4 Sublethal effects (KCA 8.3.1.4)

Sub-lethal effects on honey bees were assessed in cage, tunnel and field tests. Please refer to the CP part of the dossier under points CP 9.5.1.5 and CP 9.5.1.6.

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

The toxicity of the active substance beta-cyfluthrin to non-target arthropods was investigated by studies conducted with the representative formulations Bulldock EC 25 and Montur Forte FS 230. Please refer to the respective Volume_3CP document for the detailed description of the studies.
One extended laboratory study with larvae of the soil-dwelling arthropod *Poecilus cupreus* was conducted with the beta-cyfluthrin (technical substance).
It was cited in the dossier for Montur Forte FS 230 and not yet submitted/evaluated on EU level.
Therefore this study is described below:

KIIA 8.8.1.3

Author:	Neumann, P.
Title:	Acute effects of beta-Cyfluthrin (tech.) on larvae of carabid beetles (Poecilus cupreus) under extended laboratory test conditions
Date:	03.06.2002
Doc ID:	M-079000-02-1
Report no.:	NNP/PC006
Guidelines:	Internal testing method, Bayer AG
GLP:	yes
Validity:	not applicable; control mortality of 20 % is deemed to be too high

Material and methods:
The effect of Beta-cyfluthrin (tech.) (content of as: 98.5 %, TOX No.: 5102-00, specification: article No.: 0400838, batch No.: 380866077) on larvae of *Poecilus cupreus* was assessed to soil residues of the test substance. The soil substrate (LUFA 2.1) was moistened to about 40 % of it's water holding capacity. Test application rates were 0.005, 0.010 and 0.015 mg beta-cyfluthrin/kg soil (dry weight),

respectively. The whole study duration was 55 days. Endpoints were mortality (individuals which fail to hatch successfully), development time (time to metamorphosis), and adult body weight. The reference treatment caused a mortality rate of 100 %.

Findings:

Test species	***Poecilus cupreus* (larval stages)**			
Exposure	natural standard soil (Lufa 2.1)			
Test formulation	Control	beta-Cyfluthrin		
Application rate	---	0.005 mg/kg	0.010 mg/kg	0.015 mg/kg
Mortality rate [%]	20	25	0	10
Time to metamorphosis [%]	43.0	52.7	42.9	45.1
Mean adult body mass [mg]	89.4	81.2	81.7	79.7*

*statistically significant different from the control treatment

Conclusion:
An exposure to beta-cyfluthrin at 0.005 and at 0.010 mg/kg had no effect on mortality of the larvae or body mass of the hatched beetles. However, a significantly reduced body mass was determined at 0.015 mg/kg .

Therefore, the NOEC =0.010 mg/kg soil (dw) and the $ER_{50} \geq 0.015$ mg/kg soil (dw).

B.9.4 Effects on non-target soil meso- and macrofauna

B.9.4.1 Earthworm – sub-lethal effects

An acute study on *Eisenia fetida* with beta-cyfluthrin (technical substance) was submitted and evaluated in the course of the initial Annex I inclusion of beta-cyfuthrin. This study (Heimbach, 1987) is classified as valid and summarised shortly below.
New chronic reproduction studies on *Eisenia fetida* with the beta-cyfluthrin representative formulations Bulldock EC 25 and Montur Forte FS230 were conducted and are summarised in the corresponding Vol3 CP B9 document.
For the main soil metabolites FPB-acid and DCVA, two acute studies on *Eisenia fetida* and two reproduction studies are available and summarised below.

Table B.9.4-1: Toxicity of beta-cyfluthrin and metabolites FPB-acid and DCVA to earthworms

Species	**Test design**	**LC50 (mg as/kg dw soil)**	**NOEC (mg as/kg dw soil)**	**Reference**	**reliability**
Beta-Cyfluthrin					
Eisenia fetida	14 d acute	>1000 >500 [1]	10 5 [1]	KIIA 8.9.1/01 HBF/RG 83 Heimbach, 1987 M-053564-01-1 R-19143	valid
FPB-acid					
Eisenia fetida	14 d acute	> 63 > 31.5 [1]	< 63 <31.5[1]	KIIA 8.9.1/02 09P11RA Moser and Scheffczyk, 2009 M-354192-01-1 R-27979	valid

Eisenia fetida	56 d chronic	-	5.2 (reproduction) **2.6 (reproduction) [1]**	KIIA 8.9.2/01 kra/Rg-R-143/13 Kratz, 2013a M-468873-01-1 R-34697	valid
DCVA					
Eisenia fetida	14 d acute	122.7 61.35 [1]	< 63 <31.5 [1]	KIIA 8.9.1/03 09P10RA Moser, 2009 M-356435-01-1 R-27978	valid
Eisenia fetida	56 d chronic	184.76 92.38 [1]	5.2 (reproduction) **2.6 (reproduction) [1]**	KIIA 8.9.2/02 kra/Rg-R-157/13 Kratz, 2013b M-468552-01-1 R-34696	valid

Studies shaded in grey have been reviewed as part of the 2002 EU evaluation.
Values in bold: Endpoints used for risk assessment
[1] endpoint corrected/divided with a factor of 2, due to log Pow >2 and peat content of 10 % in study
log Pow results FPB-acid at 23 °C: Log Pow = 2.6 at pH 5, Log Pow = 0.8 at pH 7, Log Pow = -0.5 at pH 9
log Pow results DCVA at 25 °C: Log Pow = 2.5 at pH 5, Log Pow = 0.8 at pH 7, Log Pow = -0.8 at pH 9

KIIA 8.9.1/01

Author:	Heimbach, F.
Title:	Acute toxicity of FCR 4545 (techn.) to earthworms
Date:	03.09.1987
Doc ID:	M-053564-01-1
Report no.:	HBF/RG 83
Guidelines:	OECD Guideline No. 207 (OECD Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests, 4 April 1984)
GLP:	yes
Validity:	valid

Test material: Beta-cyfluthrin (FCR 4545), purity: 98.0 %, batch no. 16001/87

Results: The LC_{50} (test duration: 14 days, test species: *Eisenia foetida*) is >1000 mg as/kg dry weight concentration (NOEC) is 10 mg as/kg dry weight substrate. No mortalities were observed also at the highest concentration tested (1000 mg as/kg dry weight substrate).
Conclusion:
LC_{50} > 1000 mg as/kg dry soil.
LC_{50} (divided by 2 due to log Pow > 2) > 500 mg/askg dry soil
As data requirement have changed, this endpoint is not longer used for the risk assessement.

KIIA 8.9.1/02 (newly submitted with the dossier)

Author:	Moser, T., Scheffczyk, A.
Title:	Beta-Cyfluthrin FPB-acid: Acute toxicity to the earthworm *Eisenia fetida* in an artificial soil test
Date:	21.08. 2009
Doc ID:	M-354192-01-1
Report no.:	09P11RA
Guidelines:	OECD Guideline No. 207

GLP:	yes
Validity:	valid

Executive Summary

In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed in a for 14 days to five test concentrations of FPB-acid in artificial soil containing 10 % sphagnum peat and observed for mortality and growth. A negative control group was maintained concurrently. Four replicate test chambers for the test item and the control were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 63, 125, 250, 500 and 1000 mg test item/kg dry soil. After 14 days, number and weight of surviving adult worms was determined.
No mortality was observed at the control and at the concentration of 63 mg test item/kg soil dry weight (dw). At the concentrations of 125, 250, 500 and 1000 mg test item/kg artificial soil (dw) 85 to 100 % mortality was observed. Concerning mortality statistical analysis (Fisher's Exact Binomial Test; 1-sided; $p \leq 0.05$) reveal a significant difference between the control and the concentration s of 125, 250, 500 and 1000 mg test item/kg soil (dw). Statistical analysis (Student-t test; 2-sided, $p \leq 0.05$) showed a significant difference in the biomass development of individual adults after 14 days between the control and the concentration of the test item tested showing no mortality (i.e. 63 mg/kg soil (dw)).

The calculated 14-day LC_{50} for earthworms (*Eisenia fetida*) exposed to FPB-acid in an artificial substrate was determined to be 103.5 mg test item/kg dry soil. The corresponding no-mortality concentration was 63 mg/kg dry soil. The corresponding NOEC was determined to be < 63 mg test item/kg dry soil due to effects on biomass change.
However, due to 85 % mortality at the second treatment concentration, the calculated 14-day LC_{50} is not deemed to be a reliable endpoint. Thus, the LC_{50} is > 63 mg/kg dry soil.
As the peat content of the used artificial soil was 10 % and the log Pow of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the LC_{50} > 31.5 mg as/kg soil, NOEC mortality is 31.5 mg as/kg soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Beta-cyfluthrin FPB-acid
Description:	White needles
Lot/Batch#:	M23458
Purity	94 %

2. Vehicle and/or positive control: Chloroacetamide

3. Test organisms:

Species:	Earthworm (*Eisenia fetida*)
Age:	adults with clitellum
Weight:	300-600 mg
Source:	In house bred
Diet/Food:	none
Acclimatisation:	At least 24 h

4. Environmental conditions:

Temperature:	18-22 °C
Photoperiod:	24 h light (445 – 663 lux)
pH:	5.7 – 6.1 (start) 5.9 – 6.1 (end)
Water content:	53.1 – 58.5 % (start) 51.4 – 54.0 % (end)

Composition of artificial soil:	10 % sphagnum peat 20 % kaolin clay 1 % calcium carbonate 69 % quartz sand Deionised water

B. STUDY DESIGN

1. Experimental treatments
Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 207, 10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 69 % industrial quartz sand and 1 % calcium carbonate). Four replicate test chambers were maintained in each treatment and for the controls, with 10 worms in each test chamber. Nominal test concentrations of 63, 125, 250, 500 and 1000 mg test item/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to 50 ±10 % dry weight using deionised water. Negative control soil was treated with deionised water only.
In a separate study, earthworms were exposed to the toxic reference substance chloroacetamide. Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.

2. Observations
Mortality and mean body weights: The earthworms were exposed to the test item for 2 weeks and counted and weighed per replicate at the beginning and after 7 and 14 days of exposure. Behavioural changes: behavioural changes and morphological alterations were recorded after 7 and 14 days.

3. Statistical calculations
The LC_{50} was determined using Probit Analysis with linear max. likelihood regression. Fisher's Exact Binominal Test ($p \leq 0.05$, one-sided) was used to determine significant differences in the mean mortality of earthworms after 14 days between each concentration and the control. For biomass, treatment means were compared by ANOVA and Student t-test and tested for statistically significant ($p \leq 0.05$) differences compared to control. The statistical software package ToxRat Professional 2.10 was used for these calculations.

II. RESULTS AND DISCUSSION

A. FINDINGS
The LC_{50} and NOEC values are given below based on nominal concentrations.

Endpoints	FPB-acid (mg/kg dry soil)
LC_{50} (14 d)	> 63
NOEC (14 d) mortality	63
LOEC (14 d) mortality	125
NOEC (14 d) biomass	< 63
LOEC (14 d) biomass	≤ 63

B. OBSERVATIONS
No mortality was observed at the control and at the concentration of 63 mg/kg soil dry weight (dw). At the concentrations of 125, 250, 500 and 1000 mg test item/kg artificial soil (dw) 85 to 100 % mortality was observed, respectively. Statistical analysis (Fisher's Exact Binomial Test; $p \leq 0.05$, one-sided) revealed a significant difference between the control and the concentrations of 125, 250, 500 and 1000 mg/kg soil (dw). Statistical analysis (Student-t test; 2-sided, $p \leq 0.05$) showed a significant difference in the biomass development of individual adults after 14 days between the control and the concentration of the test item tested showing no mortality (i.e. 63 mg/kg soil (dw)).

Table 9.4-2: Effects of FPB-acid on Mortality and Biomass of *Eisenia fetida* after 14 days of exposure

Concentration (mg/kg dry soil)	Mortality (%)		Biomass (% of initial weight)
	Day 7	Day 14	Day 14
Control	0.0	0.0	114.9
63	0.0	0.0	76.7**
125	67.5	85.0*	64.7***
250	97.5	97.5*	65.7***
500	100.0	100.0*	-
1000	100.0	100.0*	-

*significantly different when compared to the control ($\alpha = 0.05$)
**significantly different to control (student-t test; $p \leq 0$-05, 2-sided)
***no statistical analysis for biomass performed since mortality was 85 % and 97.5 %

The LC_{50} for the reference test item was determined to be 50.2 mg/kg dry soil.
The mortality in the control treatments did not exceed 10 %. The validity criteria according to guideline OECD 207 are therefore fulfilled.

III. CONCLUSIONS

The calculated 14-day LC_{50} for earthworms (*Eisenia fetida*) exposed to FPB-acid in an artificial substrate was determined to be 103.5 mg test item/kg dry soil. The corresponding no-mortality concentration was 63 mg/kg dry soil. The corresponding NOEC was determined to be < 63 mg/kg dry soil due to effects on biomass change.
However, due to 85 % mortality at the second treatment concentration, the calculated 14-day LC_{50} is not deemed to be a reliable endpoint. Thus, the LC_{50} is > 63 mg/kg dry soil.
As the peat content of the used artificial soil was 10 % and the log P_{ow} of the metabolite is > 2, endpoints have to be divided by 2 before used for risk assessment.
Accordingly, the LC_{50} > 31.5 mg as/kg soil, NOEC mortality is 31.5 mg as/kg soil.

KIIA 8.9.1/03 (newly submitted with the dossier)

Author:	Moser, T.,
Title:	Beta-Cyfluthrin Permethric-acid: Acute toxicity to the earthworm *Eisenia fetida* in an artificial soil test
Date:	25.09. 2009
Doc ID:	M-356435-01-1
Report no.:	09P10RA
Guidelines:	OECD Guideline No. 207
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed in a for 14 days to five test concentrations of permethric acid (DCVA) in artificial soil containing 10 % sphagnum peat and observed for mortality and growth. A negative control group was maintained concurrently. Four replicate test chambers for the test item and the control were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 63, 125, 250, 500 and 1000 mg test item/kg dry soil. After 14 days, number and weight of surviving adult worms was determined.
No mortality was observed at the control and 2.5 % at the concentration of 63 mg test item/kg soil dry weight (dw). At the concentrations of 125 mg test item/kg artificial soil (dw) 50 % mortality and at the concentrations of 266, 500 and 1000 mg test item/kg artificial soil (dw) 100 % was observed was observed. Concerning mortality statistical analysis (Fisher's Exact Binomial Test; 1-sided; $p \leq 0.05$) revealed a significant difference between the control and the concentrations of 125, 250, 500 and 1000 mg test item/kg soil (dw). Statistical analysis (Student-t test; 2-sided, $p \leq 0.05$) showed a significant

difference in the biomass development of individual adults after 14 days between the control and the concentration of the test item tested showing less than 10 % mortality (i.e. 63 mg/kg soil (dw)).

The 14-day LC_{50} for earthworms (*Eisenia fetida*) exposed to permethric acid (DCVA) in an artificial substrate was determined to be 122.7 mg test item/kg dry soil. The corresponding no-mortality concentration was 63 mg/kg dry soil. The corresponding NOEC was determined to be < 63 mg/kg dry soil due to effects on biomass change.
As the peat content of the used artificial soil was 10 % and the log P_{ow} of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the LC_{50} > 61.35 mg as/kg soil, NOEC mortality is < 31.5 mg as/kg soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Beta-cyfluthrin Permethric-acid
Description:	White crystals
Lot/Batch#:	920622ELB03
Purity	99.8 %

2. Vehicle and/or positive control: Chloroacetamide

3. Test organisms:

Species:	Earthworm (Eisenia fetida)
Age:	adults with clitellum
Weight:	300-600 mg
Source:	In house bred
Diet/Food:	none
Acclimatisation:	At least 24 h

4. Environmental conditions:

Temperature:	19-22 °C
Photoperiod:	24 h light (593 – 795 lux)
pH:	5.8 – 6.0 (start) 5.8 – 6.0 (end)
Water content:	55.7 – 59.8 % (start) 56.6 – 59.4 % (end)
Composition of artificial soil:	10 % sphagnum peat 20 % kaolin clay 1 % calcium carbonate 69 % quartz sand Deionised water

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 207, 10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 69 % industrial quartz sand and 1 % calcium carbonate). Four replicate test chambers were maintained in each treatment and for the controls, with 10 worms in each test chamber. Nominal test concentrations of 63, 125, 266, 500 and 1000 mg test item/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted to 50 ±10 % dry weight using deionised water. Negative control soil was treated with deionised water only.

In a separate study, earthworms were exposed to the toxic reference substance chloroacetamide. Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.

2. Observations
Mortality and mean body weights: The earthworms were exposed to the test item for 2 weeks and counted and weighed per replicate at the beginning and after 7 and 14 days of exposure. Behavioural changes: behavioural changes and morphological alterations were recorded after 7 and 14 days.

3. Statistical calculations
The LC_{50} was determined using Probit Analysis with linear max. likelihood regression. Fisher's Exact Binominal Test ($p \leq 0.05$, one-sided) was used to determine significant differences in the mean mortality of earthworms after 14 days between each concentration and the control. For biomass, treatment means were compared by ANOVA and Student t-test and tested for statistically significant ($p \leq 0.05$) differences compared tot he control. The statistical software package ToxRat Professional 2.10 was used for these calculations.

II. RESULTS AND DISCUSSION

A. FINDINGS
The LC_{50} and NOEC values are given below based on nominal concentrations.

Endpoints	FPB-acid (mg/kg dry soil)
LC_{50} (14 d)	122.7
NOEC (14 d) mortality	63
LOEC (14 d) mortality	125
NOEC (14 d) biomass	< 63
LOEC (14 d) biomass	≤ 63

B. OBSERVATIONS
No mortality was observed at the control at the concentration of 63 mg/kg soil dry weight (dw) the mortality was 2.5 %. At the concentration of 125 mg/kg artificial soil (dw) 50 % mortality and at the concentrations of 266, 500 and 100 mg test item/kg artificial soil (dw) 100 % mortality was observed. Statistical analysis (Fisher's Exact Binomial Test; 1-sided; $p \leq 0.05$) reveal a significant difference between the control and the concentrations of 125, 266, 500 and 1000 mg/kg soil (dw). Statistical analysis (Student-t test; 2-sided, $p \leq 0.05$) showed a significant difference in the biomass development of individual adults after 14 days between the control and the lowest concentration of the test item tested showing less than 10 % mortality (i.e. 63 mg/kg soil (dw)).

Table 9.4-3: Effects of FPB-acid on Mortality and Biomass of *Eisenia fetida* after 14 days of exposure

Concentration (mg/kg dry soil)	Mortality (%)		Biomass (% of initial weight)
	Day 7	Day 14	Day 14
Control	0.0	0.0	115.3
63	0.0	2.5	79.9**
125	7.5	50.0*	70.6***
250	100.0	100.0*	-
500	100.0	100.0*	-
1000	100.0	100.0*	-

*significantly different to the control (Fisher's Exact Binominal Test, 1-sided; $p \leq 0.05$)
**significantly different to control (student-t test; $p \leq$ 0-05, 2-sided)
***no statistical analysis for biomass performed since mortality was 50 %

The LC_{50} for the reference test item was determined to be 45.09 mg/kg dry soil.
The mortality in the control treatments did not exceed 10 %. The validity criteria according to guideline OECD 207 are therefore fulfilled.

III. CONCLUSIONS

The 14-day LC_{50} for earthworms (*Eisenia fetida*) exposed to Permethric-acid in an artificial substrate was determined to be 122.7 mg/kg dry soil. The corresponding no-mortality concentration was 63 mg test item/kg dry soil. The corresponding NOEC was determined to be < 63 mg/kg dry soil due to effects on biomass change.
As the peat content of the used artificial soil was 10 % and the log Pow of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the LC_{50} is 61.35 mg as/kg soil, NOEC mortality is < 31.5 mg as/kg soil.

KIIA 8.9.2/01 (newly submitted with the dossier)

Author:	Kratz, M.-A.
Title:	Beta-Cyfluthrin-FPB-acid (BCS-AA52287): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Date:	27.09. 2013
Doc ID:	M-468873-01-1
Report no.:	kra/Rg-R-143/13
Guidelines:	OECD Guideline No. 222, 2004 and ISO 11268-2, 1998
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed in a 56 day test to eight test concentrations of FPB-acid in artificial soil containing 10 % sphagnum peat and observed for mortality, weight change, feeding activity and reproduction. Results from the most recent toxic standard reference test were used to show the sensitivity of the test system. Four replicate test chambers for the test item and eight replicate test chambers for the control were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 3.0, 5.2, 9.2, 16.0, 28.1, 49.0, 86.0 and 150.0 mg/kg dry soil.
Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
After 28 days of exposure one worm died in the control group, which results in a mortality rate of 1.25 %.
No mortality was observed at any test item concentration.
Statistically significant different values for the growth relative to the control were observed at all test item concentrations except 49.0 and 86.0 mg /kg soil (Williams multiple sequential t-test, 2-sided, α = 0.05). Since the weight change at all lower concentrations was higher than in the control these were not considered to be an adverse effect.
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 3.0 and 5.2/kg soil. Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 9.2, 16.0, 28.1, 49.0, 86.0 and 150.0 mg/kg soil (Williams multiple sequential t-test, 1-sided, α = 0.05).

The No Observed Effect Concentration (NOEC) for growth of the earthworm *Eisenia fetida* was determined to be 86 mg/kg soil.
Overall the No Observed Effect Concentration (NOEC) for reproduction was determined to be the concentration of 5.2 mg/kg soil. The results of the probit analysis for reproduction data showed that the EC_{50} could not be determined due to mathematical reasons. The EC_{10} is given at 34.08 mg/kg soil, the EC_{20} is given at 71.50 mg/kg soil.
As the peat content of the used artificial soil was 10 % and the log P_{ow} of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, NOEC reproduction is 2.6 mg as/kg soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Beta-cyfluthrin FPB-acid
Description:	White solid
Lot/Batch#:	SES 10570-7-4
Purity	99.4 % (analysed)

2. Vehicle and/or positive control: Carbendazim EC 360 G

3. Test organisms:

Species:	Earthworm (*Eisenia fetida andrei*)
Age:	adults with clitellum
Weight:	400-600 mg
Source:	In house bred
Diet/Food:	animal manure
Acclimatisation:	1 day, in artificial soil, under test conditions

4. Environmental conditions:

Temperature:	18-22 °C
Photoperiod:	16 h light (400 – 800 lux): 8 hours dark
pH:	5.69 – 6.28 (start) 6.25 – 6.62 (end)
Water content:	32.04 – 33.53 % (53.47 – 57.23 % of the maximum WHC) (start) 31.60 – 33.13 % (52.40 – 56.21 % of the maximum WHC) (end)
Composition of artificial soil:	10 % sphagnum peat 20 % kaolin clay 0.45 % calcium carbonate added to adjust pH to 6.0 ± 0.5 68.55 % quartz sand Deionised water

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 222, 10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 70 % industrial quartz sand and calcium carbonate). Four replicate test chambers were maintained in each treatment and eight replicate test chambers were maintained for the control, with 10 worms in each test chamber. Nominal test concentrations of 3.0, 5.2, 9.2, 16.0, 28.1, 49.0, 86.0 and 150.0 mg/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted using deionised water. Negative control soil was treated with deionised water only.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.
Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.

2. Observations

Assessment of adult worm mortality, behavioural effects and biomass development was carried out

after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of off-spring) was assessed after additional 28 days (assessed 56 days after application). Mortality, weight change, feeding activity and reproduction rate were determined.

3. Statistical calculations

For the mortality data, due to mathematically reasons the LC50 could not be calculated.
For body weight change and reproduction data the homogeneity of variances was checked by Cochran's test and the normal distribution of the data was tested by Kolmogorov-Smirnov test (α = 0.05). Data were statistically evaluated by means of a Williams multiple sequential t-test (multiple comparison, two-sided for weight and one-sided smaller for reproduction, α = 0.05). The EC_{50} was determined using Probit Analysis. The statistical software package ToxRatPro Version 2.10@ was used for the calculation.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

After 28 days of exposure one worm died in the control group, which resulted in a mortality rate of 1.25 % and no mortality was observed at any test item concentration. Due to mathematically reasons the LC_{50} could not be calculated.
Statistically significant different values for biomass change relative to the control were observed, with the exception of 49.0 and 86.0 mg/kg soil (results of a Williams multiple sequential t-test, two-sided, α = 0.05). However, the statistically significant higher biomass increase of earthworms at 3.0, 5.2, 9.2, 16.0, and 28.1 mg/kg soil relative to the control is considered as not test item treatment related. Thus, the NOEC related to growth is considered to be 86 mg/kg soil and the LOEC is considered to be 150.0 mg/kg soil.
No statistically significant different values for the number of juveniles per test vessel relative to the control was observed at the test item concentrations of 3.2 and 5.2 mg/kg soil. Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the all higher test item concentrations up to 150 mg/kg soil (results of a Williams multiple sequential t-test, onesided smaller, α = 0.05). The results of the Probit Analysis for reproduction data showed that the EC_{50} could not be determined due to mathematical reasons. The EC_{10} is given at 34.08 mg/kg soil, the EC_{20} is given at 71.50 mg/kg soil (95 % confidence limit, 0.53 – 202.0 and 21.14 – 92528.8).

Table B.9.4-4: Effect of FPB-acid on earthworms (*Eisenia fetida*) in a 56-day reproduction study

FPB-acid [mg/kg soil dry weight]	**Con-trol**	**3.0**	**5.2**	**9.2**	**16.0**	**28.1**	**49.0**	**86.0**	**150**
Mortality of adult earthworms [%] after 28 days	1.25	0	0	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	31.53	35.29 *	40.25 *	40.26 *	44.11 *	40.36 *	36.38	39.78	23.19 *
Standard Deviation	7.07	5.05	6.42	5.83	6.54	7.02	6.33	3.34	5.10
Mean number of offspring per test vessel after 56 days **	506.9	461.0	472.0	453.8 **	390.5 **	412.0 **	352.3 **	268.3 **	38.3** *
Standard Deviation	54.3	44.0	31.7	18.4	42.1	41.8	56.2	53.5	24.3
Coefficient of variance (%)	10.7	9.5	6.7	4.1	10.8	10.1	16.0	19.9	63.5
% of control	-	90.9	93.1	89.5	77.0	81.3	76.4	56.8	8.4
Endpoints							**Reproduction**		
NOEC [mg/kg dry weight soil]							**5.2**		
EC_{10} [mg/kg dry weight soil 1)] (95 % confidence limits)							**34.08** (0.53 – 202.2)		
EC_{20} [mg/kg dry weight soil 1)] (95 % confidence limits)							**71.50** (21.14 – 92528.8)		
EC_{50} [mg/kg dry weight soil 1)] (95 % confidence limits)							**n.d**		

* statistical significance compared to the control (Williams Multiple Sequential t-test, two-sided, α = 0.05)
** statistical significance compared to the control (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05)
1) Probit analysis
n. d. not determined due to mathematical reasons or inappropriate data

With a control mortality of 1.25 % this validity criterion was met. The number of juvenile worms per replicate was 455 to 619 and thus this validity criterion was met. The coefficient of variance of the reproduction was 10.7 % and thus this validity criterion was met. All validity criteria according to guideline OECD 222 are therefore fulfilled.
In the most recent test with the reference item Carbendazim EC 360 G performed two months before the present study (Study No.: Rg-R-Ref 19/12; Report No. kra-Rg-R-Ref 19/12; NON-GLP, performed September 21 to November 28, 2012), there were statistically significant effects on reproduction at a concentration of 2.50 mg carbendazim/kg soil and higher; the EC_{50} for reproduction was calculated as 3.54 mg carbendazim/kg soil. These results show the sensitivity of the test animals.

III. CONCLUSIONS

The No Observed Effect Concentration (NOEC) for growth of the earthworm *Eisenia fetida* was determined to be 86 mg/kg soil FPB-acid.
Overall the No Observed Effect Concentration (NOEC) for reproduction was determined to be the concentration of 5.2 mg/kg soil. The results of the probit analysis for reproduction data show that the EC_{50} could not be determined due to mathematical reasons. The EC_{10} is given at 34.08 mg/kg soil, the EC_{20} is given at 71.50 mg/kg soil.
As the peat content of the used artificial soil was 10 % and the log P_{ow} of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, NOEC reproduction is 2.6 mg as/kg soil.

KIIA 8.9.2/02

Author:	Kratz, M.-A.
Title:	Beta-Cyfluthrin-permethric acid (BCS-AA53389): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil
Date:	27.09. 2013
Doc ID:	M-468552-01-1
Report no.:	kra/Rg-R-157/13
Guidelines:	OECD Guideline No. 222, 2004 and ISO 11268-2, 1998
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, adult earthworms (*Eisenia fetida*) were exposed in a 56 day test to eight test concentrations of permethric acid (DCVA) in artificial soil containing 10 % sphagnum peat and observed for mortality, weight change, feeding activity and reproduction. Results from the most recent toxic standard reference test were used to show the sensitivity of the test system. Four replicate test chambers for the test item and eight replicate test chambers for the control were maintained in each treatment with 10 worms in each test chamber. Nominal test concentrations were 3.0, 5.2, 9.2, 16.0, 28.1, 49.0, 86.0 and 150.0 mg/kg dry soil. Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).
After 28 days of exposure no mortality was observed in the control group. In the treatment group 9.20 mg/kg soil in sum 2 worms died (one worm each in two replicates). No mortality was observed at any other test item concentration, including the highest test concentration of 150 mg/kg soil in this study. Due to mathematically reasons the LC_{50} could not be calculated.
No statistically significant different values for biomass change relative to the control were observed

(Williams multiple sequential t-test, two-sided, α = 0.05).
No statistically significant different values for the number of juveniles per test vessel relative to the control was observed at the test item concentrations of 3.0 and 5.2 mg/kg soil. Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at all higher test item concentrations up to 150 mg/kg soil (results of a Williams multiple sequential t-test, one-sided smaller, α = 0.05).

The No Observed Effect Concentration (NOEC) for growth of the earthworm *Eisenia fetida* was determined to be ≥150 mg/kg soil permethric acid.
Overall the No Observed Effect Concentration (NOEC) for reproduction was determined to be theconcentration of 5.2 mg/kg soil. The EC_{10} is given at 31.37 mg/kg soil, the EC_{20} is given at 57.66 mg/kg soil and for the EC_{50} a value of 184.76 was calculated.
As the peat content of the used artificial soil was 10 % and the log Pow of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the NOEC reproduction is 2.6 mg as/kg soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Beta-cyfluthrin-permethic acid
Description:	White solid
Lot/Batch#:	SES 10129-2-2
Purity	99.6 % (analysed)

2. Vehicle and/or positive control: Carbendazim EC 360 G

3. Test organisms:

Species:	Earthworm (*Eisenia fetida andrei*)
Age:	adults with clitellum
Weight:	360-560 mg
Source:	In house bred
Diet/Food:	animal manure
Acclimatisation:	1 day, in artificial soil, under test conditions

4. Environmental conditions:

Temperature:	18-22 °C
Photoperiod:	16 h light (400 – 800 lux): 8 hours dark
pH:	5.79 – 5.89 (start) 6.31 – 6.45 (end)
Water content:	31.83 – 32.83 % (53.07 – 55.56 % of the maximum WHC) (start) 31.77 – 33.23 % (52.92 – 56.57 % of the maximum WHC) (end)
Composition of artificial soil:	10 % sphagnum peat 20 % kaolin clay 0.45 % calcium carbonate added to adjust pH to 6.0 ± 0.5 68.55 % quartz sand Deionised water

B. STUDY DESIGN

1. Experimental treatments

Clitellate adult earthworms were exposed to the test substance in an artificial soil substrate (OECD 222, 10 % sphagnum peat, air dried, finely ground; 20 % kaolin clay, approximately 70 % industrial quartz sand and calcium carbonate). Four replicate test chambers were maintained in each treatment and eight replicate test chambers were maintained for the control, with 10 worms in each test chamber. Nominal test concentrations of 3.0, 5.2, 9.2, 16.0, 28.1, 49.0, 86.0 and 150.0 mg/kg dry soil were thoroughly mixed into the soil substrate. The water content was adjusted using deionised water. Negative control soil was treated with deionised water only.
In a separate study, earthworms were exposed to the toxic reference substance carbendazim.
Temperature and light intensity were monitored continuously. Water content and pH were determined at the beginning and the end of the test.

2. Observations

Assessment of adult worm mortality, behavioural effects and biomass development was carried out after 28 days exposure of adult worms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application). Mortality, weight change, feeding activity and reproduction rate were determined.

3. Statistical calculations

For the mortality data, due to mathematically reasons the LC_{50} could not be calculated.
For body weight change and reproduction data the homogeneity of variances was checked by Cochran's test and the normal distribution of the data was tested by Kolmogorov-Smirnov test (α = 0.05). Data were statistically evaluated by means of a Williams multiple sequential t-test (multiple comparison, two-sided for weight and one-sided smaller for reproduction, α = 0.05). The EC_{50} was determined using Probit Analysis. The statistical software package ToxRatPro Version 2.10@ was used for the calculation.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

After 28 days of exposure no worms died in the control group. Mortality was observed at the test item concentration of 9.2 mg/kg soil (in two replicates 1 worm each).
Due to mathematically reasons the LC_{50} could not be calculated.
No statistically significant different values for the growth relative to the control were observed (Williams multiple sequential t-test, 2-sided, α = 0.05). Therefore, the NOEC related to growth is considered to be ≥150 mg/kg soil and the LOEC is considered to be >150.0 mg/kg soil.
No statistically significant different values for the number of juveniles per test vessel relative to the control was observed at the test item concentrations of 3.0 and 5.2 mg/kg soil. Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at all higher test item concentrations up to 150 mg/kg soil (Williams multiple sequential t-test, 1-sided smaller, α = 0.05). The EC_{10} is given at 31.37 mg/kg soil, the EC_{20} is given at 57.66 mg/kg soil and for the EC_{50} a value of 184.76 was calculated (95 % confidence limits: 0.21 – 113.12, 12.04 - 2586.09 and 65.53 – 427882752.00 respectively).

Table B.9.4-5: Effect of permethric acid on earthworms (*Eisenia fetida*) in a 56-day reproduction study

FPB-acid [mg/kg soil dry weight]	**Control**	**3.0**	**5.2**	**9.2**	**16.0**	**28.1**	**49.0**	**86.0**	**150**
Mortality of adult earthworms [%] after28 days	0	0	0	5	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	19.93	18.17	23.31	24.76	23.01	21.90	21.73	21.07	18.21
Standard Deviation	4.20	0.97	4.06	1.18	2.36	6.89	3.66	7.15	5.76

Mean number of offspring per test vessel after 56 days **	500.9	486.0	475.8	403.5**	428.3**	389.5**	396.3	214.0**	23.5**
Standard Deviation	44.3	33.5	35.7	38.9	17.5	46.3	83.8	37.8	9.6
Coefficient of variance (%)	8.8	6.9	7.5	9.6	4.1	11.9	21.1	17.7	40.7
% of control	-	97.0	95.0	80.6	85.5	77.8	79.1	42.7	4.7
Endpoints							**Reproduction**		
NOEC [mg/kg dry weight soil]							**5.2**		
EC_{10} [mg/kg dry weight soil 1)] (95 % confidence limits)							**31.37** (0.21 - 113.12)		
EC_{20} [mg/kg dry weight soil 1)] (95 % confidence limits)							**57.66** (12.04 – 2586.09)		
EC_{50} [mg/kg dry weight soil 1)] (95 % confidence limits)							**184.76** (65.53 – 427882752.00)		

* statistical significance compared to the control (Williams Multiple Sequential t-test, two-sided, α = 0.05)
** statistical significance compared to the control (Williams Multiple Sequential t-test, one-sided smaller, α = 0.05)
1) Probit analysis

With a control mortality of 0 % this validity criterion was met. The number of juvenile worms per replicate was 406 to 535 and thus this validity criterion was met. The coefficient of variance of the reproduction was 8.8 % and thus this validity criterion was met. All validity criteria according to guideline OECD 222 are therefore fulfilled.
In the most recent test with the reference item Carbendazim EC 360 G performed two months before the present study (Study No.: Rg-R-Ref 19/12; Report No. kra-Rg-R-Ref 19/12; NON-GLP, performed September 21 to November 28, 2012), there were statistically significant effects on reproduction at a concentration of 2.50 mg carbendazim/kg soil and higher; the EC50 for reproduction was calculated as 3.54 mg carbendazim/kg soil. These results show the sensitivity of the test animals.

III. CONCLUSIONS

The No Observed Effect Concentration (NOEC) for growth of the earthworm *Eisenia fetida* was determined to be ≥150 mg /kg soil permethric acid.
Overall the No Observed Effect Concentration (NOEC) for reproduction was determined to be the concentration of 5.2 mg/kg soil. The EC_{10} is given at 31.37 mg/kg soil, the EC_{20} is given at 57.66 mg/kg soil and for the EC_{50} a value of 184.76 was calculated.
As the peat content of the used artificial soil was 10 % and the log Pow of the metabolite is > 2, endpoints have to be devided by 2 before used for risk assessment.
Accordingly, the NOEC reproduction is 2.6 mg as/kg soil.

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

New studies have been conducted exposing *Hypoaspis aculeifer* and *Folsomia candida* to beta-cyfluthrin. In addition, studies on *Hypoaspis aculeifer* and *Folsomia candida* with the metabolites FPB-acid and DCVA (permethric-acid) are available and summarised below.

All studies summarised below were conducted in artificial soil with a peat content of 5 % or in LUFA 2.1. soil with an assumed peat content of < 5 %.
For this reason, the resulting endpoints for beta-cyfluthrin (log P_{ow} = 5.9) and its metabolites FPB-acid (log P_{ow} = 2.6) and DCVA (log P_{ow} = 2.5) are not divided by 2.
Rational:

RMS acknowledges the decision by EFSA during the peer review 91 of penflufen from April 2012 regarding the division of endpoints for lipophilic substances conducted with 5 % organic matter (peat) in the test soil. However, RMS would not support the decision to divide the endpoint of the soil macro-organisms studies performed in a standard soil with an OM content of 5 % by 2. The approach of dividing endpoints is published in the old Guidance document (GD) for terrestrial ecotoxicology (SANCO/10329/2002). By the time the old GD was discussed, tests for soil organisms were conducted with testing soils containing 10 % peat only. Therefore, a division of endpoints was necessary to address the bioavailability of chemicals for soil organisms in test soils. Assuming a linear correlation between the OM-content and the bioavailability of chemicals for test organisms, it would be inconsistent to divide endpoints by 2 from tests conducted with soils containing 5 % OM.
A linear correlation between OM-content and bioavailability for soil organisms provided, tests conducted with soils containing 10 % OM had to be divided by 4 if tests conducted with soils containing 5 % OM are divided by two.

Moreover, if the division of endpoints by two is used for both OM-contents (5 % + 10 %), there would not be an incentive for applicants to submit new studies on soil organisms with soils containing 5 % OM anymore.

This question should urgently be clarified by an eligible committee on EU-level, independentlyfrom the evaluation of active substances according to regulation EC No 1107/2009.
Unless the further approach how to handle tests with different OM-content in testing soils is not clear, tests on soil organisms with 5 % OM in testing soils should not be divided by two.

Table B.9.4-6: Effect of beta-cyfluthrin and metabolites FBP-acid and DCVA to soil macroorganisms other than earthworms

Species	Test design	NOEC (reproduction) (mg as/kg dry soil)	Reference	reliability
Beta-Cyfluthrin				
Hypoaspis aculeifer	14 d chronic	**0.97**	KIIA 8.9.2/03 74501089 Pavic, 2012 M-476271-01-1; R-30149	valid
Folsomia candida	28 d chronic	**56**	KIIA 8.9.2/04 FRM-Coll-172/14 Frommholz, 2014 M-475305-01-1; R-34698	valid
FPB-acid				
Hypoaspis aculeifer	14 d chronic	**297**	KIIA 8.9.2/05 P14HR Moser and Scheffczyk, 2005a M-258697-01-1; R-23564	valid
Folsomia candida	28 d chronic	**28**	KIIA 8.9.2/06 FRM-Coll-144/12 Frommholz, 2012a M-440962-01-1; R-34695	valid

DCVA				
Hypoaspis aculeifer	14 d chronic	**≥ 316** **100 (mortality)**	KIIA 8.9.2/07 P15HR Moser and Scheffczyk, 2005b M-259607-01-1; R-23565	valid
Folsomia candida	28 d chronic	**18**	KIIA 8.9.2/08 FRM-Coll-143/12 Frommholz, 2012b M-440379-01-1; R-34694	valid

Values in bold: Endpoints used for risk assessmen

KIIA 8.9.2/03 (newly submitted with the dossier)

Author:	Pavic, B.
Title:	Effects of Beta-Cyfluthrin on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil with 5 % Peat
Date:	17.12.2012
Doc ID:	M-476271-01-1
Report no.:	4501089
Guidelines:	OECD Guideline No. 226, 2008
GLP:	yes
Validity:	valid

Deviations: No major deviations (water content was not checked on day 7 after application by re-weighing additional test containers)

Executive Summary

In the laboratory study the toxicity and reproductive inhibition of beta-cyfluthrin to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 0.30, 0.54, 0.97, 1.8, 3.2, 5.7, 10.2 and 18.4 mg/kg dry soil. In addition a blank control with deionised water and a toxic reference (BAS 152 11 I) were tested. 40 mites (10/ test unit) per test concentration and 80 mites per control (10/ test unit) were put in a glass bottle on artificial soil with incorporated test item. Adults and juveniles were counted after 14 d. The test item beta-cyfluthrin caused no statistically significant mortality of adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Reproduction of the predatory mites exposed to beta-cyfluthrin was not statistically significantly different compared to the control up to and including the test concentration of 0.97 mg/kg soil. At the test concentrations of 1.8 mg/kg soil and higher, reproduction was statistically significantly reduced (Williams t-test, $\alpha = 0.05$, one-sided smaller). All validity criteria according to the guidelines were fulfilled.

The overall No Observed Effect Concentration (NOEC) was determined to be 18.4 mg beta-cyfluthrin/kg soil (for mortality) and 0.97 mg beta-cyfluthrin/kg soil (for reproduction). The overall Lowest Observed Effect Concentration (LOEC) was determined to be 1.8 mg beta-cyfluthrin/kg soil and the EC_{50} for reproduction was determined to be 9.47 mg beta-cyfluthrin/kg soil (95 % confidence limits of 7.03 to 14.18 mg/kg artificial soil dry weight). The EC_{20} and EC_{10} for reproduction were determined to be 2.97 mg beta-cyfluthrin/kg soil (95 % confidence limits of 1.51 to 4.25 mg/kg artificial soil dry weight) and 1.62 mg beta-cyfluthrin/kg soil (95 % confidence limits of 0.58 to 2.64 mg/kg artificial soil dry weight), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Beta-cyfluthrin
Description:	White solid
Lot/Batch#:	PNBC000623
Purity	Analysed: 99.3 % w/w

2. Vehicle and/or positive control:	Reference item: BAS 152 11 I (Dimethoate, 400 g/L (nominal))

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites (female)
Source:	Cultured by IBACON
Diet/Food:	One spatula of cheese mite (*Tyrophagus putrescentiae* cultured by IBACON) at experimental start and on day 2, 4, 7, 9 and 11.

4. Environmental conditions:

Temperature:	18-22 °C
Composition of artificial soil:	5 % sphagnum peat 20 % kaolin clay 0.3 % calcium carbonate approx. 74.7 % quartz sand Deionised water
Water content:	At test start: 20.7 % to 21.0 % (54.4 % to 55.2 % of the maximum water holding capacity) At test end: 19.6 % to 21.6 % (51.5 % to 56.8 % of the maximum water holding capacity)
pH:	Test start: 6.4 Test end: 6.4 – 6.5
Light cycle:	16 h light : 8 h dark

B. STUDY DESIGN

1. Experimental treatments

Beta-cyfluthrin was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at eight application rates, equivalent to 0.30, 0.54, 0.97, 1.8, 3.2, 5.7, 10.2 and 18.4 mg/kg dry soil. In addition, a blank control with deionised water and a toxic reference [BAS 152 11 I (Dimethoate, 400 g/L)] were tested.

Each test item concentration was tested with 40 mites (4 replicates and 10 adult females per test unit), while the control group consisted of 8 replicates.

The mites were put in glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight with the requested test item concentrations and closed. All vessels including the additional containers were ventilated on days 2, 4, 7, 9 and 11 by opening the lids for a short period.

2. Observations

Water content and pH were determined at test start and after 14 days. Number of surviving adult female predatory mites 14 days after test initiation was recorded. Missing adult predatory mites were

recorded as dead as it was assumed they would have died and degraded during the test period.
The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Number of juvenile mites at day 14 after application was counted after extraction.

3. Statistical calculations
Mortality data were statistically analysed using Fisher's Exact test (α = 0.05, one-sided greater).
Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Cochran´s test (α = 0.05). As data were normally distributed and homogeneous, the further statistical evaluation was performed using Williams t-test (multiple comparison, α = 0.05, one-sided smaller).
The determination of the NOEC and LOEC values was based on the results of the statistical evaluation. The EC_{50}, EC_{20} and EC_{10} were calculated by Probit analysis (Finney, 1971). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

II. RESULTS AND DISCUSSION

A. FINDINGS
The ECx and NOEC values are given below based on nominal concentrations.

Endpoints	Beta-Cyfluthrin [mg/kg dry soil]
NOEC (mortality)	18.4
LC_{50} (mortality)	>18.4
NOEC (reproduction)	0.97
EC_{50} (reproduction)[1)]	9.47
EC_{20} (reproduction)[1)]	2.97
EC_{10} (reproduction)[1)]	1.62

1) Probit analysis

B. OBSERVATIONS
A mortality of up to 23 % was observed in the highest test item treated group, which was not statistically significantly different compared to the control, where 9 % of the adult mites died (Fisher's Exact Test, α = 0.05, one-sided greater).
Reproduction of the predatory mites exposed to beta-cyfluthrin was not statistically significantly different compared to the control up to and including the test concentration of 0.97 mg/kg soil. At the test concentrations of 1.8 mg/kg soil and higher, reproduction was statistically significantly reduced (Williams t-test, α = 0.05, one-sided smaller).

Table B.9.4-7: Effect of beta-cyfluthrin on the predatory mite *Hypoaspis aculeifer* in a 14-day reproduction study

FPB-acid [mg/kg soil dry weight]	Control	0.30	0.54	0.97	1.8	3.2	5.7	10.2	18.4
Mortality (day 14) [%]	9	8	10	8	3	8	18	8	23
Statistical significance 1)	-	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
No. of juveniles (day 14)	284	290	294	258	229	242	203	111	95
Reproduction in [%] of control (day 14)	-	102	103	91	81	85	72	39	34
Statistical		n.s	n.s	n.s	*	*	*	*	*

significance 2)									

n.s. = not significantly different compared to the control
* = significantly different compared to the control
- = not applicable
1) Fisher's Exact Test, α = 0.05, one-sided greater
2) Williams t-test, α = 0.05, one-sided smaller

Reference test
Treatment with the reference item Dimethoate, 400 g/L resulted in a NOEC of 1.0 mg as/kg soil for mortality and reproduction. The reference item dimethoate showed statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil and above. The EC_{50} for reproduction was 4.0 mg dimethoate/kg soil.
Validity
All validity criteria for the study were met, as adult mortality in the control treatments did not exceed 20 %, the mean number of juveniles per test unit was > 50 in the control at test end and the coefficient of variation (CoV) of the control reproduction was < 30 %.

III. CONCLUSIONS

The overall No Observed Effect Concentration (NOEC) was determined to be 18.4 mg beta-cyfluthrin/kg soil (for mortality) and 0.97 mg beta-cyfluthrin/kg soil (for reproduction).

The overall Lowest Observed Effect Concentration (LOEC) was determined to be 1.8 mg beta-cyfluthrin/kg soil and the EC_{50} for reproduction was determined to be 9.47 mg beta-cyfluthrin/kg soil (95 % confidence limits of 7.03 to 14.18 mg/kg artificial soil dry weight). The EC_{20} and EC_{10} for reproduction were determined to be 2.97 mg beta-cyfluthrin/kg soil (95 % confidence limits of 1.51 to 4.25 mg/kg artificial soil dry weight) and 1.62 mg beta-cyfluthrin/kg soil (95 % confidence limits of 0.58 to 2.64 mg/kg artificial soil dry weight), respectively.

KIIA 8.9.2/04 (newly submitted with the dossier)

Author:	Frommholz, U.
Title:	Beta-Cyfluthrin as: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Date:	28.01.2014
Doc ID:	M-475305-01-1
Report no.:	FRM-Coll-172/14
Guidelines:	OECD Guideline No. 232, 2009
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, ten collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed in a 28 day test to eight concentrations of beta-cyfluthrin.The treatment rates were 3.2, 5.6, 10, 18, 32, 56, 100 and 178 mg/kg soil. The tests were performed at 20 ± 2 °C, 400 – 800 lux, 16h light: 8h dark. During the study, they were fed with granulated dry yeast.
The vessels were briefly opened twice a week for aeration. Water content was checked 14 days after application and the vessels were rewetted with the approximately 2-fold amount of the missing water. Mortality and reproduction were determined after 28 days. The adult and juvenile Collembola of each vessel were counted using digital image on screen. All validity criteria according to the guidelines were fulfilled.
In the Collembola reproduction study with beta-cyfluthrin a NOEC of 56 mg/kg soil (dw) was determined. The LOEC was 100 mg/kg soil (dw).

I. MATERIALS AND METHODS

A. MATERIALS

1. Testmaterial:

Test item:	Beta-cyfluthrin
Description:	White solid
Lot/Batch#:	PNBC000693
Purity	Analysed: 99.1 % w/w

2. Vehicle and/or positive control:	Boric acid, test concentrations: 44, 67, 100, 150 and 225 mg Boric acid/kg soil (dw)

3. Test organisms:

Species:	*Folsomia candida (*Collembola, Isotomidae*)*
Age:	9 – 12 days old
Source:	Cultured by Bayer CropScience
Diet/Food:	2 - 10 mg granulated dry yeast at the start of the test and after 14 days

4. Environmental conditions:

Temperature:	20 ± 2 °C
Composition of artificial soil:	5 % sphagnum peat 20 % kaolin clay Calcium carbonate ($CaCO_3$) for the adjustment to pH 6.0±0.5 approx. 75 % fine quartz sand Deionised water
Soil water content:	At test start: 20.86 % to 22.11 % (44.81 % to 48.25 % of the maximum water holding capacity) At test end: 21.19 % to 22.63 % (45.73 % to 49.74 % of the maximum water holding capacity)
pH:	Test start: 6.17 – 6.26 Test end: 6.26 – 6.44
Light intensity:	400 – 800 Lux
Light cycle:	16 h light : 8 h dark

B. STUDY DESIGN

1. Experimental treatments

Beta-cyfluthrin was evaluated for mortality and reproductive reduction in a test with *Folsomia candida* at eight application rates, e.g. 3.2, 5.6, 10, 18, 32, 56, 100 and 178 mg/kg dry soil. In addition a blank control with deionised water was tested.
Each test item concentration was tested with 40 collembola (4 replicates and 10 collembola per test unit) in the control 80 collembola (8 replicates and 10 collembola per test unit).
The collembola were put in glass vessels (volume: 140 mL; diameter: 5 cm), covered with plastic lids, filled with approximately 30 g artificial soil wet weight with the requested test item concentrations and closed. The vessels were briefly opened twice a week for aeration.

2. Observations

Light intensity, water content and pH were determined at test start and after 28 days. Numbers of surviving adult and living juveniles were counted 28 days after application. Water content was checked

14 days after application and the vessels were rewetted with the approximately 2-fold amount of the missing water. Mortality and reproduction were determined after 28 days.

3. Statistical calculations
Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's -Test ($\alpha = 0.05$) respectively.
Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's-t test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values.
The software used to perform the statistical analysis was ToxRat Professional 2.10 released February 20, 2010.

II. RESULTS AND DISCUSSION

A. FINDINGS
The NOEC value is given below based on nominal concentrations.

Endpoints	Beta-Cyfluthrin [mg/kg dry soil]
NOEC (reproduction)	56
LOEC (reproduction)	100

B. OBSERVATIONS
In the control group 16.3 % of the adult *Folsomia candida* died. Meaningful EC_{10} and EC_{20} values could not be determined due to statistical reasons.

Table B.9.4-8: Effect of beta-cyfluthrin on *Folsomia candida*

Beta-Cyfluthrin [mg/kg soil dry weight] nominal concen-tration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)	Significance (*)
Control	16.3	1212.0 ± 165.8		
3.2	25.0	970.3 ± 192.5	80.1	-
5.6	5.0	1121.8 ± 80.0	92.6	-
10	27.5	1204.3 ± 160.4	99.4	-
18	17.5	1038.5 ± 177.1	85.7	-
32	15.0	1233.3 ± 112.0	101.8	-
56	10.0	6.0 ± 239.8	95.4	-
100	12.5	997.3 ± 79.5	82.3	*
178	22.5	853.3 ± 149.7	70.4	*

The calculations were performed with un-rounded values
(*) = (William's-t test one-sided-smaller, $\alpha = 0.05$, + = significant, - = not significant)

Reference test
Boric acid showed an EC_{50} of 108 mg test item/kg soil (dw) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 67 mg Boric acid/kg soil (dw) and accordingly the $LOEC_{reproduction}$ is 100 mg Boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, $\alpha = 0.05$, one-sided smaller.
This shows that the test organisms are sufficiently sensitive.

Validity
All validity criteria for the study were met, as adult mortality in the control treatments did not exceed 20 %, the mean number of juveniles per test unit was > 100 in the control at test end and the coefficient of variation (CoV) of the control reproduction was <30 %.

III. CONCLUSIONS

The NOEC for reproduction of *Folsomia candida* was 56 mg/kg soil dw beta-cyfluthrin and the LOEC reproduction was 100 mg /kg soil dw.

KIIA 8.9.2/05 (newly submitted with the dossier)

Author:	Moser, T.; Scheffczyk, A.
Title:	Beta-Cyfluthrin FPB-acid: Effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer* CANESTRINI (Acari: Laelapidae) in standard soil (LUFA 2.1)
Date:	12.10. 2005
Doc ID:	M-258697-01-1
Report no.:	P14HR
Guidelines:	SECOFASE, Final Report, improvement and standardisation of test systems for assessing sublethal effects of chemicals on fauna in the soil ecosystem (Løkke & van Gestel 1996), Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al*. 1994).
GLP:	yes
Validity:	valid

Deviations: None, the study is valid according to current OECD Guideline No. 226

Executive Summary

In the laboratory study the toxicity and reproductive inhibition of FPB-acid to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 9.4, 30.1, 94.0, 297.0 and 940.0 mg/kg dry soil. In addition a blank control with deionised water and a toxic reference (Perfekthion) were tested.
80 mites (20/ test vessel) per test concentration and 100 mites per control (20/ test vessel) were put in a glass bottle on artificial soil with incorporated test item. Adults and juveniles were counted after 14 d. The test item FPB-acid caused no statistically significant mortality of adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. A significant difference concerning the cumulative number of juveniles per female after 7 days between the control females and the females of the concentration of 940.0 mg test item/kg soil (dw) was observed. All validity criteria according to the guidelines were fulfilled.

In conclusion, in a 14 d laboratory test to determine the effects of FPB-acid on the predatory mite, *Hypoaspis aculeifer*, the 14-d EC_{50} could not be determined (> 940 mg test item/kg dry soil). The $NOEC_{mortality}$ was ≥ 940 mg test item/kg dry soil and the $NOEC_{reproduction}$ was 297 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin FPB-acid
Description:	White needles
Lot/Batch#:	M23458
Purity	Analysed: 94 % w/w

2. Vehicle and/or positive control:	Reference item: Perfekthion (Dimethoate, EC 400)

3. Test organisms:

Species:	*Hypoaspis aculeifer* (Canestrini)
Age:	Adult mites
Source:	The culture in this test is kept at ECT Oekotoxikologie GmbH since February 2002. The organisms were originally delivered by MITOX Laboratories (Amsterdam, The Netherlands).
Diet/Food:	The mites were fed with prey mites (Tyrophagus putrescentiae) or enchytraeids (Enchytraeus luxuriosus). Food was checked twice per week and was added on demand.

4. Environmental conditions:

Temperature:	24.2 – 24.9 °C
Composition of artificial soil:	LUFA 2.1 (A peat content of < 5 % is assumed.)
Soil water content:	Test start: 14.6 – 17.4 % (42.2 – 50.4 % of WHC)
pH:	Test start: 4.9 – 5.4
Light cycle:	16 h light : 8 h dark

B. STUDY DESIGN

1. Experimental treatments

FPB-acid was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at five application rates, equivalent to 9.4, 30.1, 94.0, 297.0 and 940.0 mg/kg dry soil. In addition a blank control with deionised water and a toxic reference (Perfekthion (Dimethoate, EC 400)) were tested.

Each test item concentration was tested with 80 mites (20/ test vessel), while the control group consisted of 5 replicates and the reference item of 3 replicates.

The mites were put in glass containers (able to be closed tightly) of about 30 mL capacity containing 5.1 – 5.3 g (fresh weight) artificial soil with the requested test item concentrations and closed, but opened for food supply and check of humidity of the test substrate. Two weeks after introducing the test organisms the parental and juvenile were counted.

2. Observations

Water content and pH were determined at test start. Adult and juvenile mites were counted at test end.

3. Statistical calculations

The statistical analysis was performed with the software ToxRat Professional 2.09. A One-Way

Analysis of Variance (ANOVA), followed by a Dunnett's t-test (1-sided, $p \leq 0.05$) was used to determine whether or not there were significant differences in the mean mortality of mites after 14 days between each concentration and the control. The Welch t-test for inhomogeneous variances (1-sided, $p \leq 0.05$) was used to determine whether or not there were significant differences in the mean cumulative total number of juveniles (= fertile eggs)/female/7days between the control females and the females of the two highest concentrations causing less than 50 % mortality. Abbott's formula was used to correct for control mortality.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value and the NOEC are given below based on nominal concentrations.

Endpoints	FPB-acid (mg/kg dry soil)
NOEC mortality	≥940
NOEC reproduction	297
LC_{50}/EC_{50}	could not be calculated ≥940

B. OBSERVATIONS

The test item FPB-acid caused no statistically significant mortality (ANOVA and Dunnett's t-test (1-sided, $p \leq 0.05$)) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period. Statistical analysis (Welch t-test; 1-sided, $p \leq 0.05$) showed a significant difference concerning the cumulative number of juveniles per female after 7 days between the control females and the females of the concentration of 940.0 mg test item/kg soil (dw).

Table B.9.4-9: Effects on Mortality and Reproduction of *Hypoaspis aculeifer*

Concentration [mg test item/kg dry soil]	Average mortality (%)	Corrected mortality [1] [%]	Mean cumulative number of juveniles/female after 7 days [%]	Reduction of juveniles [%]
Control	3.0	-	21.95 ± 6.26	
9.4	11.25	8.51	-	-
30.1	6.25	3.35	-	-
94.0	5.00	2.06	-	-
297.0	16.25	13.66	20.11 ± 5.53	8.40
940.0	12.50	9.76	15.50 ± 4.32 *	29.38

[1] calculated with Abbott 1925
* significantly different to control (Welch t-test; 1-sided, $p \leq 0.05$)

Reference test

Treatment with the reference item Perfekthion (Dimethoate, EC 400) at a concentration of 5 mg as/ kg dry soil resulted in an average mortality of 96.67 % (corrected mortality: 96.56 %).

Validity

All validity criteria were fulfilled, as adult mortality in the control treatments did not exceed 25 %, the mean number of juveniles per female was > 10 at test end and the adult mortality in the reference treatments was between 50 – 99.5 %.

III. CONCLUSIONS

In a 14 d laboratory test to determine the effects of FPB-acid on the predatory mite, *Hypoaspis aculeifer*, the 14-d EC_{50} could not be determined (>940 mg test item/ kg dry soil). The $NOEC_{mortality}$ was ≥ 940 mg test item/kg dry soil and the $NOEC_{reproduction}$ was 297 mg test item/kg dry soil.

KIIA 8.9.2/06 (newly submitted with the dossier)

Author:	Frommholz, U.
Title:	Beta-Cyfluthrin-FPB acid (BCS-AA52287): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Date:	05.11. 2012
Doc ID:	M-440962-01-1
Report no.:	FRM-Coll-144/12
Guidelines:	OECD Guideline No. 232, 2009
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, ten collembola (10-12 days old) per replicate were exposed in a 28 day test to one (1st run) and 3 (2nd run) concentrations of FPB acid. Since the first run on the test item did not provide a final result, a second run test run was performed using lower test concentrations. In the 1st test run 10 collembolans per replicate (8 replicates for the control group and for the treatment group) were exposed to control (water treated) and 100 mg test item/kg soil. In the 2nd test run 10 collembolans per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 28, 51 and 90 mg test item/kg soil. Both runs were performed at 20 ± 2 °C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast. The vessels were briefly opened twice a week for aeration. Water content was checked 14 days after application and the vessels were rewetted with the approximately 2-fold amount of the missing water. Mortality and reproduction were determined after 28 days. The adult and juvenile Collembola of each vessel were counted using digital image on screen. All validity criteria according to the guidelines were fulfilled.

In the collembola reproduction study with FPB acid a LC_{50} could not be calculated and is considered to be >100 mg test item/kg soil (dw). For reproduction a NOEC of 28 mg test item/kg soil (dw) was determined. The LOEC was 51 mg test item/kg soil (dw).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin-FPB acid (BCS-AA52287)
Description:	White solid
Lot/Batch#:	SES 10570-7-4
Purity	Analysed: 99.4 % w/w

2. Vehicle and/or positive control:	Boric acid, test concentrations: 44, 67, 100, 150 and 225 mg Boric acid/kg soil (dw)

3. Test organisms:

Species:	*Folsomia candida* (Collembola, Isotomidae)
Age:	10 – 12 days old
Source:	Cultured by Bayer CropScience
Diet/Food:	2 mg granulated dry yeast at the start of the test and after 14 days

4. Environmental conditions:

Temperature:	Within 18 - 22 °C
Composition of artificial soil:	5 % Sphagnum-peat 20 % Kaolin clay Calcium carbonate (CaCO3) for the adjustment to pH 6.0±0.5 approximately 75 % fine quartz-sand
Soil water content:	At test start: 1st test run: 21.44 % (48.09 % of the maximum water holding capacity) 2nd test run: 19.72 % to 20.11 % (46.93 % to 48.09 % of the maximum water holding capacity) At test end: 1st run 21.09 % to 21.33 % (47.11 % to 47.78 % of the maximum water holding capacity) 2nd test run: 19.20 % to 20.37 % (45.37 % to 48.84 % of the maximum water holding capacity)
pH:	Test start: 5.96 – 5.99 (1st test run), 5.63 – 6.06 (2nd test run) Test end: 5.81 – 5.86 (1st test run), 5.98 – 6.03 (2nd test run)
Light intensity:	400 – 800 Lux
Light cycle:	16 h light : 8 h dark

B. STUDY DESIGN

1. Experimental treatments

FPB acid was evaluated for mortality and reproductive reduction in a test with *Folsomia candida* at four application rates, e.g. 100 (1st test run), 90, 51 and 28 mg test item/kg dry soil (2nd test run). In addition a blank control with deionised water was tested. Since the first run on the test item did not provide a final result, a second run test run was performed using lower test concentrations.
Each test item concentration was tested with 80 collembola (8 replicates and 10 collembola per test unit) in the 1st test run and 40 collembola (4 replicates and 10 collembola per test unit) in the 2nd test run.
The collembola were put in glass vessels (volume: 140 mL; diameter: 5 cm, height: 7 cm), covered with plastic lids, filled with approximately 30 g artificial soil wet weight with the requested test item concentrations and closed. The vessels were briefly opened twice a week for aeration.

2. Observations

Light intensity, water content and pH were determined at test start and after 28 days. Numbers of surviving adult and living juveniles were counted 28 days after application. Water content was checked 14 days after application and the vessels were rewetted with the approximately 2-fold amount of the missing water. Mortality and reproduction were determined after 28 days.

3. Statistical calculations

Data of reproduction of both test runs were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's -Test ($\alpha = 0.05$) respectively.
Because data of reproduction were normally distributed and variances were homogenous Student-t test (1st test run) and William's test (2nd run) were used to determine NOEC and LOEC values (one sided smaller, $\alpha = 0.05$).
The software used to perform the statistical analysis was ToxRat Professional 2.10 released February 20, 2010.

II. RESULTS AND DISCUSSION

A. FINDINGS

The NOEC value is given below based on nominal concentrations.

Endpoints	FPB-acid (mg/kg dry soil)
NOEC reproduction	28
LOEC reproduction	51

Table B.9.4-10: Effect of FPB acid on *Folsomia candida*

FPB-acid (mg/kg dry soil) nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
1st run			
Control	1.3	1167.4 ± 101.4	-
100	7.5	1067.8 ± 76.9	91.5 *
2nd run			
Control	3.8	1169.1 ± 99.1	
90	5.0	1046.3 ± 120.3	89.5 *w
51	15.0	1022.5 ± 96.3	87.5 *w
28	8.0	1208.8 ± 138.3	103.4 n s

The calculations were performed with un-rounded values
SD = Standard deviation
* = statistically significant (Student-t test one-sided-smaller, α = 0.05)
*w = statistically significant William's test, one-sided-smaller, α = 0.05)
n.s. = statistically not significant (William's test, one-sided-smaller, α = 0.05)

Reference test
Boric acid showed an EC_{50} of 116 mg test item/kg soil (dw) for reproduction according probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 67 mg boric acid/kg soil (dw) and accordingly the $LOEC_{reproduction}$ is 100 mg boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, α = 0.05, one-sided smaller.
This shows that the test organisms are sufficiently sensitive.
Validity
All validity criteria for the study were met, as adult mortality in the control treatments did not exceed 20 %, the mean number of juveniles per test unit was > 100 in the control at test end and the coefficient of variation (CoV) of the control reproduction was <30 %.

III. CONCLUSIONS

In the collembola reproduction study with FPB acid a LC_{50} could not be calculated and is considered to be > 100 mg test item/kg soil (dw). For reproduction a NOEC of 28 mg test item/kg soil (dw) was determined. The LOEC was 51 mg test item/kg soil (dw).

KIIA 8.9.2/07 (newly submitted with the dossier)

Author:	Moser, T.; Scheffczyk,A.
Title:	Beta-Cyfluthrin Permethric-acid: Effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer* CANESTRINI (Acari: Laelapidae) in standard soil (LUFA 2.1)
Date:	27.10. 2005b

Doc ID:	M-468552-01-1
Report no.:	P15HR
Guidelines:	SECOFASE, Final Report, improvement and standardisation of test systems for assessing sublethal effects of chemicals on fauna in the soil ecosystem (Løkke & van Gestel 1996), Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett *et al*. 1994)
GLP:	yes
Validity:	valid

Executive Summary
In the laboratory study the toxicity and reproductive inhibition of permethric-acid to *Hypoaspis aculeifer* was tested. Adult mites were exposed to 10, 32, 100, 316 and 1000 mg test item/kg dry soil. In addition a blank control with deionised water and a toxic reference (Perfekthion) were tested.
80 mites (20/ test vessel) per test concentration and 100 mites per control (20/ test vessel) were put in a glass bottle on artificial soil with incorporated test item. Adults and juveniles were counted after 14 d. The test item beta-cyfluthrin permethric-acid caused statistically significant difference in mortality of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period between the control and the two highest concentrations (316 and 1000 mg test item/kg soil (dw)) of the test item tested. No significant difference concerning the cumulative number of juveniles per female after 7 days between the control females and the females of the concentration of 100 and 316 mg test item/kg soil (dw) was observed. All validity criteria according to the guidelines were fulfilled.

In conclusion, in a 14 d laboratory test to determine the effects of permethric-acid on the predatory mite, *Hypoaspis aculeifer*, the 14-d LC_{50} was 400.9 mg test item/ kg dry soil. The $NOEC_{mortality}$ was 100 mg test item/kg dry soil and the $NOEC_{reproduction}$ was ≥316 mg test item/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin Permethric acid (the test item was a 1:1 mixture of the a) cis- and b) trans-isomer)
Description:	a) White crystals b) White powder
Lot/Batch#:	a) 920622ELB03 b) 920622ELB04
Purity	a) analysed: 99.8 % w/w b) analysed: 99.8 % w/w

2. Vehicle and/or positive control: Reference item: Perfekthion (Dimethoate, EC 400, 392.1 g/L analysed)

3. Test organisms:

Species:	*Hypoaspis aculeifer (Canestrini)*
Age:	Adult mites
Source:	The culture in this test is kept at ECT Oekotoxikologie GmbH since February 2002. The organisms were originally delivered by MITOX Laboratories (Amsterdam, The Netherlands).
Diet/Food:	The mites were fed with prey mites (*Tyrophagus*

	putrescentiae) or enchytraeids (*Enchytraeus luxuriosus*). Food was checked twice per week and was added on demand.

4. Environmental conditions:

Temperature:	24.2 – 25.3 °C
Composition of artificial soil:	LUFA 2.1 (A peat content of < 5 % is assumed.)
Soil water content:	Test start: 15.2 – 16.4 % (41.6 – 44.8 % of WHC)
pH:	Test start: 4.9 – 5.4
Light cycle:	permanent dark

B. STUDY DESIGN

1. Experimental treatments

Permethric acid was evaluated for mortality and reproductive reduction in a test with *Hypoaspis aculeifer* at five application rates, equivalent to 10, 32, 100, 316 and 1000 mg test item/kg dry soil.
In addition a blank control with deionised water and a toxic reference (Perfekthion (Dimethoate, EC 400, 392.1 g/L)) were tested.
Each test item concentration was tested with 80 mites (20/ test vessel), while the control group consisted of 5 replicates and the reference item of 3 replicates.
The mites were put in glass containers (able to be closed tightly) of about 30 mL capacity containing 5.1 – 5.3 g (fresh weight) artificial soil with the requested test item concentrations and closed, but opened for food supply and check of humidity of the test substrate. Two weeks after introducing the test organisms the parental and juvenile were counted.

2. Observations

Water content and pH were determined at test start. Adult and juvenile mites were counted at test end.

3. Statistical calculations

The statistical analysis was performed with the software ToxRat Professional 2.09. A One-Way Analysis of Variance (ANOVA), followed by a Dunnett's t-test (1-sided, $p \leq 0.05$) was used to determine whether or not there were significant differences in the mean mortality of mites after 14 days between each concentration and the control. The Welch t-test for inhomogeneous variances (1- sided, $p \leq 0.05$) was used to determine whether or not there were significant differences in the mean cumulative total number of juveniles (= fertile eggs)/female/7days between the control females and the females of the concentrations of 100 and 316 mg test item/kg soil (dw) causing less than 50 % mortality. Abbott's formula was used to correct for control mortality.

II. RESULTS AND DISCUSSION

A. FINDINGS

The EC_{50} value and the NOEC are given below based on nominal concentrations.

Endpoints	FPB-acid (mg/kg dry soil)
NOEC reproduction	100
LOEC reproduction	≥316
LC_{50}/EC_{50}	400.9

B. OBSERVATIONS

Permethric acid caused statistically significant difference in mortality (ANOVA and Dunnett's t-test

(1-sided, $p \leq 0.05$)) of the adult *Hypoaspis aculeifer* at the end of the 14-day exposure period between the control and the two highest concentrations (316 and 1000 mg test item/kg soil (dw)) of the test item tested. Statistical analysis (Welch t-test; 1-sided, $p \leq 0.05$) showed no significant difference concerning the cumulative number of juveniles per female after 7 days between the control and the concentrations of 100 and 316 mg test item/kg soil (dw).

Table B.9.4-11: Effects on Mortality and Reproduction of *Hypoaspis aculeifer*

Concentration [mg test item/kg dry soil]	Average mortality (%)	Corrected mortality [1] [%]	Mean cumulative number of juveniles/female after 7 days [%]	Reduction of juveniles [%]
Control	7.0	-	24.1 ± 4.6	
10	12.5	5.9	-	-
32	6.3	-0.8	-	-
100	13.8	7.3	23.7 ± 7.0	1.9
316	30.0*	24.7*	26.4 ± 5.5	-9.3
1000	93.8*	93.3*	-	-

[1] calculated with Abbott 1925
* significantly different to control (Dunnett's t-test; 1-sided, $p \leq 0.05$)

Reference test
Treatment with the reference item Perfekthion (Dimethoate, EC 400) at a concentration of 5 mg as/ kg dry soil resulted in an average mortality of 96.7 % (corrected mortality: 96.4 %).
Validity
All validity criteria were fulfilled, as adult mortality in the control treatments did not exceed 25 %, the mean number of juveniles per female was > 10 at test end and the adult mortality in the reference treatments was between 50 – 99.5 %.

III. CONCLUSIONS

In a 14 d laboratory test to determine the effects of permethric acid on the predatory mite, *Hypoaspis aculeifer*, the 14-d LC_{50} was 400.9 mg test item/kg dry soil. The $NOEC_{mortality}$ was 100 mg test item/kg dry soil and the $NOEC_{reproduction}$ was ≥ 316 mg test item/kg dry soil.

KIIA 8.9.2/08 (newly submitted with the dossier)

Author:	Frommholz, U.
Title:	Beta-Cyfluthrin-permethric acid (BCS-AA53389): Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil
Date:	24.10.2012
Doc ID:	M-440379-01-1
Report no.:	FRM-Coll-143/12
Guidelines:	OECD Guideline No. 232, 2009
GLP:	yes
Validity:	valid

Executive Summary
In a laboratory study, ten collembola (10-12 days old) per replicate were exposed in a 28 day test to one (1st test run) and 5 (2nd test run) concentrations of permethric acid. Since the first run on the test item did not provide a final result, a second run test run was performed using lower test concentrations. In the 1st test run 10 collembolans per replicate (8 replicates for the control group and for the treatment group) were exposed to control (water treated) and 100 mg test item/kg soil. In the 2nd test run 10 collembolans per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 5.6, 10, 18, 32 and 56 mg test item/kg soil. Both

test runs were performed at 20 ± 2 °C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.
The vessels were briefly opened twice a week for aeration. Water content was checked 14 days after application and the vessels were rewetted with the approximately 2-fold amount of the missing water. Mortality and reproduction were determined after 28 days. The adult and juvenile Collembola of each vessel were counted using digital image on screen. All validity criteria according to the guidelines were fulfilled.

In the collembola reproduction study a LC_{50} could not be calculated. For reproduction a NOEC of 18 mg test item/kg soil (dw) was determined. The LOEC was 32 mg test item/kg soil (dw). The EC_{10}, EC_{20} and EC_{50} were calculated to be 18.3, 24.1 and 40.7 mg test item/kg soil (dw), respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin-permethric acid (BCS-AA53389)
Description:	White solid
Lot/Batch#:	SES 10129-2-2
Purity	Analysed: 99.6 % w/w

2. Vehicle and/or positive control:	Boric acid, test concentrations: 44, 67, 100, 150 and 225 mg Boric acid/kg soil (dw)

3. Test organisms:

Species:	*Folsomia candida* (Collembola, Isotomidae)
Age:	10 – 12 days old
Source:	Cultured by Bayer CropScience
Diet/Food:	2 mg granulated dry yeast at the start of the test and after 14 days

4. Environmental conditions:

Temperature:	Within 18 - 22 °C
Composition of artificial soil:	5 % Sphagnum-peat 20 % Kaolin clay Calcium carbonate (CaCO3) for the adjustment to pH 6.0±0.5 approximately 75 % fine quartz-sand
Soil water content:	At test start: 1st test run: 21.44 % to 22.18 % (48.09 % to 50.23 % of the maximum water holding capacity) 2nd test run: 19.72 % to 20.17 % (46.93 % to 48.25 % of the maximum water holding capacity) At test end: 1st run 21.09 % to 21.31 % (47.11 % to 47.74 % of the maximum water holding capacity) 2nd test run: 18.85 % to 20.27 % (44.35 % to 48.56 % of the maximum water holding capacity)
pH:	Test start: 5.96 – 5.94 (1st test run), 6.10 – 6.26 (2nd test run) Test end: 5.81 – 5.78 (1st test run), 5.95 – 6.03

	(2nd test run)
Light intensity:	400 – 800 Lux
Light cycle:	16 h light : 8 h dark

B. STUDY DESIGN

1. Experimental treatments

Permethric acid was evaluated for mortality and reproductive reduction in a test with *Folsomia candida* at six application rates, e.g. 100 (1st test run), 90, 51 and 28 mg test item/kg soil (2nd test run). In addition a blank control with deionised water was tested. Since the first run on the test item did not provide a final result, a second run test run was performed using lower test concentrations.
Each test item concentration was tested with 80 collembola (8 replicates and 10 collembola per test unit) in the 1st test run and 40 collembola (4 replicates and 10 collembola per test unit) in the 2nd test run.
The collembola were put in glass vessels (volume: 140 mL; diameter: 5 cm, height: 7 cm), covered with plastic lids, filled with approximately 30 g artificial soil wet weight with the requested test item concentrations and closed. The vessels were briefly opened twice a week for aeration.

2. Observations

Light intensity, water content and pH were determined at test start and after 28 days. Numbers of surviving adult and living juveniles were counted 28 days after application. Water content was checked 14 days after application and the vessels were rewetted with the approximately 2-fold amount of the missing water. Mortality and reproduction were determined after 28 days.

3. Statistical calculations

Data of reproduction of both test runs were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's -Test ($\alpha = 0.05$), respectively.
Because data of reproduction were normally distributed and variances were homogenous Welch-t test (1st test run) and William's test (2nd run) were used to determine NOEC and LOEC values (one sided smaller, $\alpha = 0.05$).
The software used to perform the statistical analysis was ToxRat Professional 2.10 released February 20, 2010.

II. RESULTS AND DISCUSSION

A. FINDINGS

The NOEC value is given below based on nominal concentrations.

Endpoints	Permethric acid [mg/kg dry soil]
EC_{10} reproduction	18.3
EC_{20} reproduction	24.1
EC_{50} reproduction	40.7
NOEC reproduction	18
LOEC reproduction	32

B. OBSERVATIONS

In the control group 1.3 % (1st run) and 3.8 % (2nd run) of the adult *Folsomia candida* died. A LC_{10}, LC_{20} and LC_{50} could not be determined.

Table B.9.4-12: Effect of permethric acid on *Folsomia candida*

Beta-Cyfluthrin-permethric acid mg test item/kg soil dry	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)

weight nominal concentration			
1st run			
Control	1.3	1167.4 ± 101.4	-
100	66.3	42.6 ± 17.4	3.7*w
2nd run			
Control	3.8	1169.1 ± 99.1	-
56	22.5	357.3 ± 105.2	30.6*
32	5.0	565.3 ± 386.8	64.7*
18	17.5	1063.5 ± 120.0	91.0n s
10	5.0	1222.3 ± 134.4	104.5n s
5.6	10.0	1041.0 ± 155.8	89.0n s
		1 Adult mortality	Reproduction

The calculations were performed with un-rounded values
SD = Standard deviation
1) Probit analysis
*w = statistically significant (Welch-t test one-sided-smaller, α = 0.05)
* = statistically significant (William's test, one-sided-smaller, α = 0.05)
n.s. = statistically not significant (Student-t test, one-sided-smaller, α = 0.05)

Reference test
Boric acid showed an EC_{50} of 116 mg test item/kg soil (dw) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be 67 mg boric acid/kg soil (dw) and accordingly the $LOEC_{reproduction}$ is 100 mg boric acid/kg artificial soil dry weight according Williams-Test multiple t-test procedure, α = 0.05, one-sided smaller.
This shows that the test organisms are sufficiently sensitive.

Validity
All validity criteria for the study were met, as adult mortality in the control treatments did not exceed 20 %, the mean number of juveniles per test unit was > 100 in the control at test end and the coefficient of variation (CoV) of the control reproduction was <30 %.

III. CONCLUSIONS

In the collembola reproduction study a LC_{50} could not be calculated. For reproduction a NOEC of 18 mg test item/kg soil (dw) was determined. The LOEC was 32 mg test item/kg soil (dw). The EC_{10}, EC_{20} and EC_{50} were calculated to be 18.3, 24.1 and 40.7 mg test item/kg soil (dw), respectively.

B.9.5 Effects on soil nitrogen transformation

In the EU evaluation of beta-cyfluthrin (2002), laboratory soil micro-organism studies were reviewed that evaluated the effect of beta-cyfluthrin on microbial activities in soil (Blumenstock, 1987 and Anderson, 1987). These tests were performed with cyfluthrin K+L (beta-cyfluthrin) and are considered to be valid. Additionally, nitrogen mineralisation studies with the two major soil metabolites (i.e. FPB-acid and DCVA) were conducted and the results are summarised below.

Table B.8.5-1: Effects on soil micro-organisms

Test design	**NOEC (reproduction) (mg as/kg dry soil)**	**Reference**	**reliability**
Beta-Cyfluthrin			
Nitrogen mineralisation 28-day study	No significant effects (>25 %) on nitrogen mineralisation by day 28 at 0.018 and **0.18**	KIIA 8.10.1/02 BSI/47987 Blumenstock,	valid

	kg/ha	1987 M-054489-01-2 R-19148	
Carbon mineralisation 28-day study	No significant effects (>25 %) on microbial respiration by day 28 at 0.018 and **0.18 kg/ha**	KIIA 8.10.1/01 AJO/46887 Anderson, 1987 M-054544-01-2 R-19147	valid
FPB-acid			
Nitrogen mineralisation 28-day study	No significant effects (>25 %) on nitrogen mineralisation at 0.012 mg/kg dry soil and 0.125 mg/kg dry soil, corresponding to 0.009 kg and 0.094 kg test item/ha, respectively	KIIA 8.10.1/03 13 10 48 016 N Schulz, 2013a M-454537-01-1 R-34704	valid
DCVA			
Nitrogen - mineralisation 28-day study	No significant effects (>25 %) on nitrogen mineralisation at 0.011 mg/kg dry soil and 0.112 mg/kg dry soil, corresponding to 0.008 kg and 0.084 kg test item/ha, respectively	KIIA 8.10.1/04 13 10 48 017 N Schulz, 2013b M-454538-01-1 R-34705	valid

Studies shaded in grey have been reviewed as part of the 2002 EU evaluation.
Values in bold: Endpoints used for risk assessment

KIIA 8.10.1/02

Author:	Blumenstock, I.
Title:	Influence of Cyfluthrin K+L (FCR 4545) on the microbial mineralisation of nitrogen in soils
Date:	17.12.1987
Doc ID:	M-054489-01-2
Report no.:	BSI/47987
Guidelines:	BBA Guideline part VI, 1-1 (1987)
GLP:	no
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 216
Test material: Beta-cyfluthrin (FCR 4545), purity: 98.3 %, batch no. 16001/87

Results: The highest recommended dosage of beta-cyfluthrin (Cyfluthrin K+L) and a 10-fold overdose (equivalent to 0.0240 and 0.2400 mg as/kg dry wt. soil or 0.018 and 0.1800 kg as/ha) had no meaningful influence on soil nitrogen mineralisation and on nitrification of added ammonium in a loamy sand soil (0.84 % org. C, pH (KC1) = 5.3) or a silt soil (1.23 % org. C, pH (KC1) = 4.8). In field soils, Cyfluthrin K+L should have no negative effects on the nitrogen cycle.

Conclusion: NOEL = 0.1800 kg as/ha

KIIA 8.10.1/01

Author:	Anderson, J. P. E.
Title:	Influence of Cyfluthrin K+L (FCR 4545 techn.) on the

	microbial mineralisation of carbon in soils
Date:	03.121987
Doc ID:	M-054544-01-2
Report no.:	AJO/46887
Guidelines:	BBA Guideline part VI, 1-1 (1987)
GLP:	no
Validity:	valid

Deviations: The study is valid according to the current OECD Guideline No. 217

Results: The recommended amount of beta-cyfluthrin (Cyfluthrin K+L) and a 10-fold overdose (equivalent to 0.0240 and 0.2400 mg as/kg dry wt. soil or 0.018 and 0.1800 kg as/ha) had no meaningful influence on soil respiration or the mineralisation of lucerne grass green meal in a loamy sand (0.84 % org. C, pH (KC1) =5.3) or a silt soil (1.23 % org. C, pH (KC1) = 4.8). Applied under practical conditions, the insecticide should have no negative influence on carbon transformations in soils.

Conclusion: NOEL = 0.18 kg as/ha

KIIA 8.10.1/03 (newly submitted with the dossier)

Author:	Schulz, L.
Title:	Beta-Cyfluthrin-FPB acid (BCS-AA52287) – Effects on the activity of soil microflora (Nitrogen transformation test)
Date:	21.05.2013
Doc ID:	M-454537-01-1
Report no.:	13 10 48 016 N
Guidelines:	OECD Guideline No. 216, 2000
GLP:	yes
Validity:	valid

Executive Summary
The effects of FPB-acid on the nitrogen transformation (NO_3-nitrogen production) was investigated in an agricultural soil at two concentrations, namely 0.012 mg/kg dry soil and 0.125 mg/kg dry soil (corresponding to 0.009 kg/ha and 0.094 kg/ha assuming a soil depth of 5 cm and a soil density of 1.5 g/cm^3). Soil samples were incubated at 18.7-21.1 °C, while stored in test vessels in the dark. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined 0, 7, 14 and 28 days after treatment.
No adverse effects of the test item on nitrogen transformation in soil were observed at both tested concentrations after 28 days.

FPB acid has no significant long-term effect on nitrogen transformation in soil at concentrations of 0.012 mg/kg dry soil and 0.125 mg/kg dry soil, corresponding to 0.009 kg/ha and 0.094 kg/ha, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin-FPB acid
Description:	White solid
Lot/Batch#:	SES 10570-7-4
Purity	99.4 % (analysed)

2. Reference item: Dinoterb (purity 98.0 % ± 0.5 analysed)

3. Test system:

Soil:	Agriculturally utilised soil
Source:	Wassergut Canitz, field “Schlag 34/3”, Saxony, Germany
Water content of soil::	8.77 g/100 g soil d.w.
pH:	6.7
Total Org. C:	1.98 %
Clay (< 0.002 mm):	10.1 % (ISO 11277) / 10.1 % (USDA)
Silt (0.002-0.063 mm(ISO 11277) / (0.002-0.050(USDA)):	36.9 % (ISO 11277) / 35.6 % (USDA)
1277) / 0.05-2.0 (USDA)):	53.1 % (ISO 11277) / 54.3 % (USDA)

4. Environmental conditions:

Temperature:	18.7-21.1 °C
pH:	6.5-6.6
Water content:	46.52-48.50 % of WHC
Illumination	Darkness

B. STUDY DESIGN AND METHODS

1. Experimental treatments

For the investigation of potential effects of the test item beta-cyfluthrin-FPB acid, nitrogen transformation (NO_3-nitrogen production) of test item treated soil was compared with a non-treated control soil. Per each replicate, 200 g soil d.w. per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial NH_4-N- and NO_3- N-content. The NO_3-N content was 1.55 mg/100 g soil d.w.. The test item was mixed with quartz meal and the obtained mixture was subsequently mixed with the soil. Two test rates of beta-cyfluthrin-FPB acid were applied: 0.012 mg test item/kg dry soil (corresponding to an application rate of 0.009 kg test item/ha) and 0.125 mg test item/kg dry soil (corresponding to an application rate of 0.094 kg test item/ha). Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40-50 % of WHC. Soil samples were incubated at 18.7-21.1 °C, while stored in test vessels in the dark.
Although not required in the method protocol, the reference item Dinoterb was tested in a separate study (R 13 10 48 001 N) at concentrations of 6.8, 16.0 and 27.0 mg/kg.

2. Observations

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH_4-N, NO_3-N and NO_2-N content were determined. For extraction, 50 mL 1 M KCl solution (10 g soil d.w. with 50 mL KCl solution) and a rotator (150 rpm) were used. The extraction duration was 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at minus 20 ± 5 °C. The analysis was performed within one week after day 28. The pHvalues of the soil were measured at test start (after application) and at the sampling on day 28, respectively.

3. Statistical calculations

Mean values per treatment, standard deviations and coefficients of variation were calculated.
A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level) and 2-sided Welch-t-test (for inhomogeneous variances at 5 % significance level).

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

The validity criterion according to guideline OECD 216 requires a variation of less than ±15 % be-

tween replicate control samples for nitrogen transformation. The coefficients of variation in the control group of the nitrogen test were maximum 3.2 % and thus fulfilled the demanded range.
Results of the nitrogen transformation test are summarised in the table below. The limits of quantification for nitrogen and ammonium were 0.05 mg/100 g soil d.w. and 0.06 mg/100 g soil d.w., respectively.

Table B.9.5-1: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	0.012 mg test item/kg soil dry weight equivalent to 0.009 kg test item/ha		0.125 mg test item/kg soil dry weight equivalent to 0.094 kg test item/ha	
	Nitrate-N[1)]	Nitrate-N[1)]	% difference to control	Nitrate-N[1)]	% difference to control
0-7	3.06 ± 0.01	2.97 ± 0.11	-2.8[n w]	2.71±0.37	-11.4[n w]
7-14	1.30 ± 0.20	1.62 ± 0.18	24.9[n s]	1.41 ± 0.33	+ 8.8[n s]
14-28	1.30 ±0.14	0.91 ±0.12	-11.4[n s]	0.98±0.11	-4.6[n s]

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n w No statistically significant difference to the control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)
n s No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

The reference item caused a stimulation of nitrogen transformation of 17.6 %, 33.7 %, and 42.6 % at 6.8, 16.0 and 27.0 mg Dinoterb per kg soil d.w., respectively, 28 days after application.

III. CONCLUSION

The study was performed in a field soil at concentrations up to 0.125 mg test item/ kg soil d.w., equivalent to 0.094 kg test item/ha. The test item caused no adverse effects (difference to control < 25 %) on the soil nitrogen transformation (measured as NO_3-N production) 28 days after application.

KIIA 8.10.1/04

Author:	Schulz, L.
Title:	Beta-Cyfluthrin-permethric acid (BCS-AA53389) – Effects on the activity of soil microflora (Nitrogen transformation test)
Date:	21.05.2013b
Doc ID:	M-454538-01-1
Report no.:	13 10 48 017 N
Guidelines:	OECD Guideline No. 216, 2000
GLP:	yes
Validity:	valid

Executive Summary
Nitrogen transformation (NO_3-nitrogen production) was compared of test item treated soil with a non-treated control soil. Three replicates were applied for the control and both test item treatments, namely 0.011 mg test item/kg dry soil (corresponding to an application rate of 0.008 kg test item/ha) and 0.112 mgtest item/kg dry soil (corresponding to an application rate of 0.084 kg test item/ha). Test concentrationsrelated to a soil depth of 5 cm and a soil density of 1.5 g/cm3. Soil samples were incubated at 18.7-21.1 °C, while stored in test vessels in the dark. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined 0, 7, 14 and 28 days after treatment.
No adverse effects of the test item on nitrogen transformation in soil were observed at both tested concentrations after 28 days.

Therefore it is concluded that permethric acid has no significant long term effecton nitrogen

transformation in soil at concentrations of 0.011 mg/kg dry soil and 0.112 mg/kg dry soil, corresponding to 0.008 kg test item/ha and 0.084 kg test item/ha, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Beta-cyfluthrin-permethric acid
Description:	White solid
Lot/Batch#:	SES 10129-2-2
Purity	99.6 % (analysed)

2. Reference item: Dinoterb (purity 98.0 % ± 0.5 analysed)

3. Test system:

Soil:	Agriculturally utilised soil
Source:	Wassergut Canitz, field “Schlag 34/3”, Saxony, Germany
Water content of soil::	8.77 g/100 g soil d.w.
pH:	6.7
Total Org. C:	1.98 %
Clay (< 0.002 mm):	10.1 % (ISO 11277) / 10.1 % (USDA)
Silt (0.002-0.063 mm(ISO 11277) / (0.002-0.050(USDA)):	36.9 % (ISO 11277) / 35.6 % (USDA)
1277) / 0.05-2.0 (USDA)):	53.1 % (ISO 11277) / 54.3 % (USDA)

4. Environmental conditions:

Temperature:	18.7-21.1 °C
pH:	6.4-6.5
Water content:	46.05-48.27 % of WHC
Illumination	Darkness

B: STUDY DESIGN AND METHODS

1. Experimental treatments

For the investigation of potential effects of the test item Permethric acid (DCVA), nitrogen transformation (NO_3-nitrogen production) of test item treated soil was compared with a non-treated control soil. Per each replicate, 200 g soil d.w. per test vessel was weighed. The soil was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without lucerne meal) was used for determination of the initial NH_4-N- and NO_3- N-content. The NO_3-N content was 1.55 mg/100 g soil d.w.. The test item was mixed with quartz meal and the obtained mixture was subsequently mixed with the soil. Two test rates of permethric acid were applied: 0.011 mg test item/kg dry soil (corresponding to an application rate of 0.008 kg test item/ha) and 0.112 mg test item/kg dry soil (corresponding to an application rate of 0.084 kg test item/ha). Water was added to the soil to achieve a water content of approximately 45 % of WHC. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40-50 % of WHC. Soil samples werincubated at 18.7-21.1 °C, while stored in test vessels in the dark.

Although not required in the method protocol, the reference item Dinoterb was tested in a separate study (R 13 10 48 001 N) at concentrations of 6.8, 16.0 and 27.0 mg/kg.

2. Observations

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH_4-N, NO_3-N and NO_2-N content were determined. For extraction, 50 mL 1 M

KCl solution (10 g soil d.w. with 50 mL KCl solution) and a rotator (150 rpm) were used. The extraction duration was 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at minus 20 ± 5°C. The pH-values of the soil were measured at test start (after application) and at the sampling on day 28, respectively.

3. Statistical calculations
Mean values per treatment, standard deviations and coefficients of variation were calculated.
A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test for homogeneous variances at 5 % significance level.

II. RESULTS AND DISCUSSION

A. FINDINGS AND OBSERVATIONS

The validity criterion according to guideline OECD 216 requires a variation of less than ±15 % between replicate control samples for nitrogen transformation. The coefficients of variation in the control group of the nitrogen test were maximum 4.3 % and thus fulfilled the demanded range.
Results of the nitrogen transformation test are summarised in the table below. The limits of quantification for nitrogen and ammonium were 0.05 mg/100 g soil d.w. and 0.06 mg/100 g soil d.w., respectively.

Table B.9.5-2: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	0.012 mg test item/kg soil dry weight equivalent to 0.009 kg test item/ha		0.125 mg test item/kg soil dry weight equivalent to 0.094 kg test item/ha	
	Nitrate-N[1)]	Nitrate-N[1)]	% difference to control	Nitrate-N[1)]	% difference to control
0-7	3.09 ± 0.28	3.26 ± 0.52	-5.2[n s]	3.14 ± 0.26	+1.9[n s]
7-14	1.77 ± 0.48	1.78 ± 0.12	+0.5[n s]	1.64 ± 0.50	-7.5[n s]
14-28	1.13 ± 0.22	1.05 ± 0.18	-7.0[n s]	1.15 ± 0.09	+1.7[n s]

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n s = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

The reference item caused a stimulation of nitrogen transformation of 17.6 %, 33.7 %, and 42.6 % at 6.8, 16.0 and 27.0 mg Dinoterb per kg soil d.w., respectively, 28 days after application.

III. CONCLUSION

The study was performed with DCVA in a field soil at concentrations up to 0.112 mg test item/ kg soil d.w., equivalent to 0.084 kg test item/ha. The test item caused no adverse effects (difference to control < 25 %) on the soil nitrogen transformation (measured as NO_3-N production) 28 days after application.

B.9.6 Effects on terrestrial non-target higher plants

Although beta-cyfluthrin is not an herbicide, a limit test to investigate possible effects on vegetative vigor and seedling emergence was performed with the representative formulation Bulldock 25 EC (please refer to Volume_3CP_Bulldock EC 25_B-9.12)

B.9.6.1 Summary of screening data

No screening data with the active substance were submitted. Studies are not required

B.9.6.2 Testing on non-target plants

Although beta-cyfluthrin is not an herbicide, a limit test to investigate possible effects on vegetative vigor and seedling emergence was performed with the representative formulation Bulldock 25 EC (please refer to Volume_3CP_Bulldock EC 25_B-9.12)

B.9.7 Effects on other terrestrial organisms (flora and fauna)

Tests on other non-target species are not required by Regulation 1107/2009.

B.9.8 Effects on biological methods for sewage treatment

A summary of the available studies with activated sludge (according to OECD 209) is below.

Table B.9.8-1: Effects of beta-cyfluthrin and cyfluthrin on biological methods of sewage treatment

Test substance Test type	Test organism	Endpoint [mg/L]	Reference
beta-Cyfluthrin (Bulldock)	activated sludge (domestic)	30 min EC_{50} >10000	CA 8.8/01 485 A/94 Caspers and Mueller, 1994a M-053009-01-2 R-34706
Cyfluthrin	activated sludge (domestic)	30 min EC_{50} >10000	CA 8.8/02 478 A/94 Caspers and Mueller, 1994b M-021811-01-1 R-19149

KIIA 8.15/01

Author:	Caspers, N.; Mueller, G.
Title:	Studies on the ecological behaviour of Bulldock
Date:	1994
Doc ID:	M-053009-01-2
Report no.:	485 A/94
Guidelines:	ISO 8192 and Official Gazette of EG L 133 Part C: Biological degradability: Examination of the respiratory inhibition (largely corresponds to the test method OECD 209)
GLP:	yes
Validity:	valid

Deviations: as the test method largely corresponds to the current OECD 209 guideline and no effects on activated sludge were observed in the highest dose rate, the study is considered valid for Annex I Renewal.
Test material: Beta-cyfluthrin technical, purity: 98.6 %, batch no. 380 466 003
Results: Test duration was 30 min and concentrations of 100, 1000 and 10000 mg/L were used. Based on the results of the measurement, a concentration of 10000 mg beta-cyfluthrin/L causes an 18.3 % inhibition of the respiration of activated sludge. The EC_{50} for the acute toxicity to bacteria was > 10000 mg/L

KIIA 8.15/02

Author:	Caspers, N.; Mueller, G.
Title:	Studies on the ecological behaviour of Cyfluthrin
Date:	1994
Doc ID:	M-021811-01-1
Report no.:	478 A/94
Guidelines:	ISO 8192 and Official Gazette of EG L 133 Part C: Biological degradability: Examination of the respiratory inhibition (largely corresponds to the test method OECD 209)
GLP:	yes
Validity:	valid

Deviations: as the test method largely corresponds to the current OECD 209 guideline and no effects on activated sludge were observed in the highest dose rate, the study is considered valid for Annex I Renewal.
Test material: Cyfluthrin technical, purity: 96.5 %, batch no. 380 368 010
Results: Based on the results of the measurement, a concentration of 10000 mg cyfluthrin/L causes an 11.2 % inhibition of the respiration of activated sludge. The EC_{50} for the acute toxicity to bacteria was > 10000 mg/L

B.9.9 Monitoring data

Monitoring data are not submitted and required.

B.9.10 Biological activity of metabolites potentially occurring in groundwater

Soil metabolites of beta-cyfluthrin do not leach into ground water.

B.9.11 References relied on

Literature research:

Search methods
A systematic literature search has been conducted in the context of the Annex I renewal of the active substance beta-cyfluthrin.
The present literature search covers the period from 1 January 2004 to 11 November 2013.
In total over 2020 titles pertaining to beta-cyfluthrin, cyfluthrin, the diastereomers and the metabolites were reviewed for their relevance and side-effects on human health, the environment and non-target species. Duplicates found in several databases were removed using the Chemical Abstract database as reference i.e., if the publication was already found in Chemical Abstract database it was considered a duplicate in the others.

Databases used for literature search
The search was conducted in the following databases

Database	Date of last database update
Agricola	2013-11-05
Biosis	2013-11-06
CABA	2013-11-06
Chemical Abstracts (CAS SciFinder)	2013-11-08
Derwent Drug File (DRUGU)	2013-11-07
EMBASE	2013-11-08

Esbiobase	2013-11-04
IPA	2013-11-04
FSTA	2013-11-04
Medline	2013-11-09
Pascal	2013-11-04
PQSciTech (LIFESCI)	2013-10-18
Registry	2013-11-08
Scisearch	2013-11-04
Toxcenter	2013-11-05
Ulidat	2013-08-14

Number of records retrieved for the relevante subtance(s) per database

Database	**Date of last database up-date**	**Parent compound**	**metabolite 4-Fluoro-3-phenoxy-benzoic acid**	**metabolite 4-Fluoro-3-phenoxy-benzalde-hyde**	**metabolite 4-Fluoro-3-phenoxybenzyl alcohol**	**metabolite Permethric acid (DCVA)**
Chemical Abstracts (Scifinder)	2013-11-08	1472	42	32	-	17
AGRICOLA	2013-11-05	28	-	-	-	1
BIOSYS	2013-11-06	121	1	1	-	1
CABA	2013-11-06	221	1	-	-	1
Derwent Drug File (DRU-GU)	2013-11-07	-	-	-	-	-
EMBASE	2013-11-04	75	-	1	1	3
ESBIOBASE	2013-11-04	5	-	-	-	-
IPA	2013-11-04	-			-	
FSTA	2013-11-04	4	-	-	-	-
Medline	2013-11-09	20	2	-	-	5
Pascal	2013-11-04	1	-	-	-	-
PQSCITECH (LIFESCI)	2013-10-18	20	-	-	-	-
SCISEARCH	2013-11-04	51	-	-	-	2
Toxcenter	2013-11-05	-	-	-	-	
Ulidat	2013-08-14	2	-	-	-	
Total number		**2020**	**46**	**34**	**1**	**30**

Results of the study selection process

The **Tier 1** search resulted in 2020 literature references for the parent compound and 111 references also cover one or more of its metabolites, after duplicates removal.
The titles identified in the Tier 1 review were further evaluated to determine the relevance for human health effects, environmental fate and behaviour and ecotoxicological impact (**Tier 2**). The output was grouped and manually screened by experts in the respective area.
It was concluded that 159 of the references (peer-reviewed and excluding patents) published over the past 10 years could be relevant for human health effects, fate and behavior of beta-cyfluthrin in the environmental and ecotoxicology. These 159 publications were further reviewed in detail (full-text) for relevance and 69 dismissed as non-relevant after the assessment. The remaining 90 peer-reviewed texts or conference procedures, for which full text was available, are listed in this section.
No relevant publication was found covering ecotoxicology.

Results[1] of the study selection process*, for each data requirement or group of data requirements Data requirement(s) captured in the search

	n
Total number of *summary records* retrieved after	2131(*)

*all** searches of peer-reviewed literature (excluding duplicates)	
Number of *summary records* excluded from the search results after rapid assessment for relevance	1972
Total number of *full-text documents* assessed in detail	159
Number of *studies* excluded from further consideration after detailed assessment for relevance of *full-text* publication	69
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	90
Number of *studies* included in the dossier	5
Number of studies excluded for which a justification is provided	85

[1]Results refer to all sections of the RAR.

The literature research concerning effects to amphibian species was repeated by the notifier. Results are presented in section B.9.1.4.
Moreover, one published study that has been excluded by the notifier was used by the RMS to assess the toxicity of the different isomers to aquatic invertebrates (Liu et al. 2005).Another study excluded by the notifier was used to determine the acute toxicity of beta-cyfluthrin to birds. Please refer to B.9.1.1.1 (KIIA8.1.1./13).

In regard to issues of ecotoxicological effect, no further studies were considered to be relevant.

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA8.1.1/01	[redacted]	1994	FCR 4545 (technical grade): Acute oral toxicity to bobwhite quail [redacted] Bayer CropScience, Report No.: VB-027, Edition Number: M-025760-01-1 Date: 1994-10-26 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.1/02	[redacted]	1985	FCR 4545 Bird toxicity oral / Japanese quail (Coturnix coturnix japonica) [redacted] Bayer CropScience, Report No.: VW-106, Edition Number: M-053473-01-2 Date: 1985-04-22 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.1/03	[redacted]	1983	Acute oral LD50 of technical Cyfluthrin to bobwhite quail [redacted] Bayer CropScience, Report No.: 426, Edition Number: M-008638-01-1 EPA MRID No.: 00131498 Date: 1983-08-15 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.1.1/04	[redacted]	1985	Acute oral toxicity (LD50) study with FCR 1272 (c.n. Cyfluthrin) vehicle: Cremophor EL 2percent in distilled water in the hen [redacted] Bayer CropScience, Report No.: R3621, Edition Number: M-039456-01-1 Date: 1985-12-31 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.1/05	[redacted]	1985	Acute oral toxicity (LD50) study with FCR 1272 (c.n. Cyfluthrin) vehicle: PEG 400 in the hen [redacted] Bayer CropScience, Report No.: R3622, Edition Number: M-039453-01-1 Date: 1985-12-31 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.1/06	[redacted]	1979	Toxcitè par voie orale pour les oiseaux/canari (Serinus canarius) [redacted] Bayer CropScience, Report No.: VK 137, Edition Number: M-030280-01-2 Date: 1979-02-22 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.1.1/07	[redacted]	1985	FCR 1272 - Vogeltoxizitaet oral / Kanarienvogel (Serinus canarius) [redacted] Bayer CropScience, Report No.: VK-253, Edition Number: M-030284-01-1 Date: 1985-04-22 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.1 /08	[redacted]	1985	FCR 4545 techn. - Study for acute oral toxicity to the chicken (gallus domesticus) [redacted] Bayer CropScience, Report No.: 13689, Edition Number: M-064864-01-1 EPA MRID No.: 41244116 Date: 1985-08-06 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.1/09	[redacted]	1980	FCR 1272 - Acute oral toxicity to quails [redacted] Bayer CropScience, Report No.: V-80518, Edition Number: M-030215-01-3 EPA MRID No.: 00143142 Date: 1980-05-29 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.1.1/10	[redacted]	**2012**	**Toxicity of Cyfluthrin technical during an acute oral LD50 with the canary (Serinus canaria)** [redacted] **BCS,** **Report No.: EBBDL009,** **Edition Number: M-442786-01-1** **EPA MRID No.: 49020801** **Date: 2012-12-03** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**data not submitted on EU Level**	**BCS-Irvita**
KIIA8.1.1/11	Addy-Orduna,L.; Zaccagnini, M-E.; Canavelli, S.B.; Mineau; P.	2011	Formulated Beta-cyfluthrin Shows Wide Divergence in Toxicity among Bird Species J. Toxicol., pp. 803451, 10 pp published	**O**	**N**		**LIT**
KIIA 8.1.2/01	[redacted]	1983	Acute dietary LC50 of Cyfluthrin technical to bobwhite quail [redacted] Bayer CropScience, Report No.: 428, Edition Number: M-008664-01-1 Date: 1983-08-18 GLP/GEP: no, unpublished	Y	N		Bayer CropScience
KIIA 8.1.2/02	[redacted]	1983	Acute dietary LC50 of Cyfluthrin technical to mallard ducks [redacted] Bayer CropScience, Report No.: 83-175-02, Edition Number: M-030228-01-2 EPA MRID No.: 00131500 Date: 1983-08-11 GLP/GEP: no, unpublished	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA8.1.4/01	[redacted]	1984	Effect of Cyfluthrin (Baythroid technical) on bobwhite quail reproduction [redacted] Bayer CropScience, Report No.: 509, Edition Number: M-030219-01-1 EPA MRID No.: 00145330 Date: 1984-08-09 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA8.1.4/02	[redacted]	1985	Effects of Cyfluthrin (Baythroid technical) on bobwhite quail eggshells [redacted] Bayer CropScience, Report No.: 654, Edition Number: M-030225-01-1 EPA MRID No.: 00152829 Date: 1985-08-07 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA8.1.4/03	[redacted]	1984	Effects of Cyfluthrin (technical Baythroid) on mallard duck reproduction [redacted] Bayer CropScience, Report No.: 508, Edition Number: M-008671-01-1 EPA MRID No.: 00145331 Date: 1984-08-09 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.1.4/04	[redacted]	1986	Baythroid Technical: A one-generation reproduction study with the mallard (Anas platyrhynchos) [redacted] Bayer CropScience, Report No.: 740, Edition Number: M-030269-01-1 EPA MRID No.: 00158782 Date: 1986-04-10 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.4/05	[redacted]	1990	Baythroid technical. A one-generation reproduction study with the mallard (Anas platyrhynchos) [redacted] Bayer CropScience, Report No.: 100359, Edition Number: M-030237-01-1 Date: 1990-09-20 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA 8.1.4/06	unkown	un-kown	A chronic study with Mallard duck: [http://www.epa.gov/espp/litstatus/effects/redleg frog/2013/Cyfluthrin/assessment.pdf]	?	?	still required	?
KII 8.1.4.	Puglis, H.J.; Boon, M.D.	2011	Effects of Technical-Grade Active Ingredient vs. Commercial Formulation of Seven Pesticides in the Presence or Absence of UV Radiation on Survival of Green Frog Tadpoles Arch.Environ. Contam. Toxicol., Volume 60, Issue 1, Page 145-155 published	O	N		LIT

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.13	**Bomann, W.**	**2014**	**Beta-cyfluthrin - Derivation of the relevant endpoint for use in the mammalian reproductive risk assessment for ecotoxicology - Expert evaluation** **Toxconsult LLC** **Irvita Plant Protection,** **Report No.: R-34692,** **Edition Number: M-483094-01-1** **Date: 2014-03-27** **GLP/GEP: no, unpublished**	**Y**	**Y**	**Higher Tier Assessment**	**Irvita Plant Protection**
KIIA8.2.1/01	[redacted]	1988	The acute toxicity of FCR 4545 technical to rainbow trout (Salmo gairdneri, Richardson) in a flow-through test [redacted] Bayer CropScience, Report No.: FF-207, Edition Number: M-056119-01-2 EPA MRID No.: 45426703 Date: 1988-06-20 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KIIA8.2.1/02	[redacted]	1994	Acute toxicity of FCR 4545 technical to rainbow trout (Oncorhynchus mykiss) under flow-through conditions [redacted] Bayer CropScience, Report No.: 103231 Edition Number: M-056053-01-1 EPA MRID No.: 45375002 Date: 1994-08-24 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA8.2.1/03	[redacted]	1994	Acute toxicity of FCR 4545 technical to bluegill (Lepomis macrochirus) under flow-through conditions [redacted] Bayer CropScience, Report No.: 103232, , Edition Number: M-056058-01-1 EPA MRID No.: 45375003 Date: 1994-08-24 GLP/GEP: yes, unpublished	Y	Y		Bayer Crop-Science
KIIA8.2.1/04	[redacted]	1988	The acute toxicity of FCR 4545 technical to golden orfe (Leuciscus idus melanotus) in a flow-through test [redacted] Bayer CropScience, Report No.: FO-1011, Edition Number: M-056152-01-2 Date: 1988-05-31 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA8.2.1/05	[redacted]	1984	Acute toxicity of Dichlorovinylcarboxylic acid to rainbow trout [redacted] Report No.: 515, Edition Number: M-034724-01-1 EPA MRID No.: 00158557 Date: 1984-09-07 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science
KIIA8.2.1/06	[redacted]	1984	Acute toxicity of Fluorphenoxybenzaldehyde to Rainbow trout [redacted] Report No.: 502, Edition Number: M-034806-01-1 EPA MRID No.: 00158554 Date: 1984-08-03 GLP/GEP: yes, unpublished	Y	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA8.2.1/07	[redacted]	**2006**	**Beta Cyfluthrin: Acute toxicity to rainbow trout** [redacted] **BCS-Irvita,** **Report No.: IRV 0134/053835,** **Edition Number: M-481575-01-1** **Date: 2006-03-10** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to identify most sensitive fish species for higher tier microsm study**	**Irvita Plant Protection**
KIIA 8.2.1/08	[redacted]	**2006**	**Beta Cyfluthrin: Acute toxicity to three-spined stickle-back** [redacted] **BCS-Irvita,** **Report No.: IRV 0123/053833,** **Edition Number: M-481578-01-1** **Date: 2006-03-10** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to identify most sensitive fish species for higher tier microsm study**	**Irvita Plant Protection**
KIIA 8.2.1/09	[redacted]	**2006**	**Beta Cyfluthrin: Acute toxicity to roach** [redacted] **BCS-Irvita,** **Report No.: IRV 0124/053834,** **Edition Number: M-481577-01-1** **Date: 2006-03-10** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to identify most sensitive fish species for higher tier microsm study**	**Irvita Plant Protection**
KIIA 8.2.1/10	[redacted]	**2006**	**Beta Cyfluthrin: Acute toxicity to fathead minnow** [redacted] **BCS-Irvita,** **Report No.: IRV 0121/053831,** **Edition Number: M-481564-01-1** **Date: 2006-03-10** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to identify most sensitive fish species for higher tier microsm study**	**Irvita Plant Protection**

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.2.1/11	[redacted]	**2006**	**Beta-cyfluthrin: Acute toxicity to bluegill sunfish [redacted]** **Irvita Plant Protection,** **Report No.: IRV 0122/053832,** **Edition Number: M-482362-01-1** **Date: 2006-03-10** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to identify most sensitive fish species for higher tier microsm study**	**Irvita Plant Protection**
KIIA 8.2.1/12	[redacted]	**2006**	**Beta Cyfluthrin: Acute toxicity to common carp [redacted]** **Irvita Plant Protection,** **Report No.: IRV 0120/053830,** **Edition Number: M-482363-01-1** **Date: 2006-03-10** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to identify most sensitive fish species for higher tier microsm study**	**Irvita Plant Protection**
KIIA 8.2.1/13	[redacted]	**2010**	**Acute toxicity of beta-Cyfluthrin FPB-acid (tech.) to fish (Oncorhynchus mykiss) under static conditions** **BCS-Irvita,** **Report No.: EBFRL003,** **Edition Number: M-364414-01-1** **Date: 2010-02-23** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**to complete the risk assessment for aquatic or-ganisms**	**BCS-Irvita**
KII 8.2.2./ KII 8.3.2	Grace, Nillos Mae, Qin Sujie, Larive Cynthia, Schlenk Daniel, Gan Jay.	2009	Epimerisation of cypermethrin stereoisomers in alcohols. Journal of agricultural and food chemistry 57 (15): 6938-43. doi:10.1021/jf900921g Published	N	N		LIT
KII 8.2.2./ KII 8.3.2	Perschke, H.; Hussain, M.	1992	Chemical isomerisation of deltamethrin in alcohols. J. Agric. Food Chem. 1992, 40, 686–690. Published	N	N		LIT

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.2.1/14; KIIA 8.3.1.1/06; KIIA 8.4/02	Hill, I. R.	1989	Aquatic organisms and pyrethroids Publisher:Society of Chemical Industry, Location:Great Britain, Journal:Pesticide Science, Volume:27, Pages:429-457, Year:1990, Report No.: Lit. 6002, Edition Number: M-090574-01-1 GLP/GEP: n.a., published	N	N		public literature
KIIA 8.2.4	[redacted]	1985	Toxicity of Cyfluthrin (Baythroid) technical to early life stages of rainbow trout [redacted] Bayer CropScience, Report No.: 683, Edition Number: M-008695-01-1 EPA MRID No.: 00155898, 40359002 Date: 1985-10-24 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KIIA 8.2.5	[redacted]	1990	Full life-cycle toxicity of 14C-Cyfluthrin (Baythroid) to the fathead minnow (pimephales promelas) under flow-through conditions [redacted] Bayer CropScience, Report No.: 100097, Edition Number: M-022913-02-1 EPA MRID No.: 41450401 Date: 1990-04-02 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.2.6.1/01	[redacted] J.	1984	Bioconcentration of Cyfluthrin (Baythroid) by bluegill sun-fish [redacted] Bayer CropScience, Report No.: 455, Edition Number: M-024032-01-1 EPA MRID No.: 00137547, 00143143 Date: 1984-01-12 GLP/GEP: no, unpublished	Y	N		Bayer Crop-Science
KIIA 8.2.6.1/02	[redacted]	**2014**	**[Fluorophenyl-14C]beta-Cyfluthrin: Bioconcentration test in the bluegill sunfish (Lepomis Macrochirus) under flow-through conditions** [redacted] **BCS-Irvita,** **Report No.: D78913,** **Edition Number: M-481021-01-1** **Date: 2014-03-03** **GLP/GEP: yes, unpublished**	**Y**	**Y**	**requested by the Rapporteur, existing study with deficiencies**	**BCS-Irvita**
KII 8.3.1	Liu, W., Gan, J. Qin, S.	2005	Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides Chirality, 17:127-133 published	N	N		LIT
KIIA 8.3.1.1/01	Forbis, A. D.	1994	Acute toxicity of FCR 4545-1 (techn.) to Daphnia magna under flow through conditions Miles Inc., Columbia, MO, USA Bayer CropScience, Report No.: 98515, Edition Number: M-056226-01-1 EPA MRID No.: 45426701 Date: 1994-08-24 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.3.1.1/02	Heimbach, F.	1988	Acute toxicity of FCR 4545 (techn.) to water fleas Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/DM 78, Edition Number: M-056188-01-2 EPA MRID No.: 45426702 Date: 1988-01-22 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
KIIA 8.3.1.1/04	Forbis, A. D.; Burgess, D.	1984	Acute toxicity of DCVA to Daphnia magna ABC Laboratories, Inc., Columbia, MO, USA BCS, Report No.: 505, Edition Number: M-034747-01-1 EPA MRID No.: 00158556 Date: 1984-06-25 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
KIIA 8.3.1.1/08, KIIA 8.3.2.1/05 Please refer to Volume 3CP_Bulldock EC 25_B-9 KIIIA1 10.2.6/01	Heimbach, F.	1999	Extended laboratory study on effects and recovery of a Daphnia magna population in a water-sediment system after application of 14C-Cyfluthrin EC 050 Xylol Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/EDM 04, Edition Number: M-041214-01-1 Date: 1999-06-07 GLP/GEP: yes, unpublished ...also filed: KCA 8.2.8 /05	N	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.3.1.1/05	Forbis, A. D.; Burgess, D.	1984	Acute toxicity of FPB ALD to Daphnia magna ABC Laboratories, Inc., Columbia, MO, USA BCS, Report No.: 504, Edition Number: M-034810-01-1 EPA MRID No.: 00158555, 00158556 Date: 1984-06-25 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
KIIA 8.3.1.1/03	**Kimmel, S.**	**2014**	**Beta-cyfluthrin: Acute toxicity to Daphnia magna in a 48-hour immobilisation test** **Harlan Laboratories Ltd., Itingen, Switzerland** **BCS-Irvita,** **Report No.: D58707,** **Edition Number: M-481046-01-1** **Date: 2014-03-19** **GLP/GEP: yes, unpublished**	**N**	**Y**	**data not submitted on EU level**	**BCS-Irvita**
KIIA 8.2.4.8/07	**Bruns, E.**	**2010**	**Acute toxicity of beta-Cyfluthrin FPB-acid (tech.) to the waterflea Daphnia magna in a static laboratory test system** **BCS-Irvita,** **Report No.: EBFRL002,** **Edition Number: M-363182-01-1** **Date: 2010-02-01** **GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for aquatic organisms**	**BCS-Irvita**
KIIA 8.3.1.3/03	Surprenant, D. C.	1987	Acute toxicity of Baythroid to mysid shrimp (Mysidopsis bahia) under flow-through conditions Springborn Laboratories, Inc., Wareham, MA, USA Bayer CropScience, Report No.: 808, Edition Number: M-027941-01-1 EPA MRID No.: 40069501 Date: 1987-01-30 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source ***(where different from company)*** Company name, Report No., Date, GLP status ***(where relevant)***, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.3.1.3/01	Machado, M. W.	1994	Acute toxicity of FCR 4545 to the mysid shrimp (Mysidopsis bahia) under flow-through conditions Springborn Laboratories, Inc., Wareham, MA, USA Bayer CropScience, Report No.: 106797, Edition Number: M-056044-01-1 EPA MRID No.: 45426709 Date: 1994-10-17 GLP/GEP: yes, unpublished	N	Y	data not submitted on EU Level	Bayer Crop-Science owner Bayer CropScience, license Irvita Plant Protec-tion B.V.
KIIA 8.3.1.3/02	Machado, M. W.	1994	Acute toxicity of FCR 4545 to the mysid shrimp (Mysidopsis bahia) under flow-through conditions Springborn Laboratories, Inc., Wareham, MA, USA Bayer CropScience, Report No.: 106588, Edition Number: M-056064-01-1 EPA MRID No.: 45426704 Date: 1994-07-19 GLP/GEP: yes, unpublished	N	Y	data not submitted on EU Level	Bayer Crop-Science owner Bayer CropScience, license Irvita Plant Protec-tion B.V.
KIIA 8.3.1.3/05; KIIA 8.3.2.1/06 Please refer to Vol-ume 3CP_Bulldock EC 25_B-9.3.2 /KIIIA1 10.2.6/01	Heimbach, F.	2000	Comparative toxicity of 14C-Cyfluthrin EC 050 to Gam-marus pulex in water and in a water sediment system under static laboratory conditions Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/SP 01-99, Edition Number: M-020399-01-1 Date: 2000-01-21 GLP/GEP: yes, unpublished ...also filed: KCA 8.2.4.1 /03	N	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.3.1.3/04	**Bradley, M. J.**	**2013**	**Cyfluthrin - Acute toxicity to freshwater amphipods (Hyalella azteca) under flow-through conditions** **Smithers Viscient, Wareham, MA, USA** **TF- Pyrethroid,** **Report No.: 13656.6168,** **Edition Number: M-458228-01-1** **EPA MRID No.: 49171201** **Date: 2013-06-24** **GLP/GEP: yes, unpublished**	**N**	**Y**	**EPA Data Call-In**	**TF- Pyrethroid**
KIIA 8.3.2.1/02	Heimbach, F.	1988	Influence of Cyfluthrin (techn.) on the reproduction rate of water fleas (Daphnia magna) Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/RDM 01, Edition Number: M-008718-01-2 Date: 1988-01-22 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KIIA 8.3.2.1/01	Forbis, A. D.	1984	Chronic toxicity of 14C-Cyfluthrin to Daphnia magna under flow-through test conditions ABC Laboratories, Inc., Columbia, MO, USA Bayer CropScience, Report No.: 557, Edition Number: M-025043-01-1 EPA MRID No.: 00151442 Date: 1984-11-07 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.3.2.1/03	**Kimmel, S.**	**2014**	**Beta-cyfluthrin: Effect on survival and reproduction of Daphnia magna in a semi-static test over three weeks Harlan Laboratories Ltd., Itingen, Switzerland BCS-Irvita, Report No.: D58718, Edition Number: M-480965-01-1 Date: 2014-03-19 GLP/GEP: yes, unpublished**	**N**	**Y**	**data not submitted on EU Level**	**BCS-Irvita**
KIIA 8.3.2.1/04	**Schwader, A. L.**	**2013**	**Beta-cyfluthrin - Life-cycle toxicity test with mysids (Americamysis bahia) Smithers Viscient, Wareham, MA, USA BCS-Irvita, Report No.: 13798.6307, Edition Number: M-465880-01-1 Date: 2013-09-18 GLP/GEP: yes, unpublished**	**N**	**Y**	**EPA Data Call-In**	**BCS-Irvita**
KIIA 8.5.2/01	Heimbach, F.	1997	Influence of beta-Cyfluthrin SC 125 on development and emergence of larvae of Chironomus riparius in a water-sediment system Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/CH 17, Edition Number: M-055336-01-1 Date: 1997-08-20 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.5.2/02	**Kimmel, S.**	**2014**	**Beta-cyfluthrin: Effect on the development of sediment-dwelling larvae of Chironomus riparius in a water-sediment system with spiked water** **Harlan Laboratories Ltd., Itingen, Switzerland** **BCS-Irvita,** **Report No.: D58720,** **Edition Number: M-481015-01-1** **Date: 2014-03-19** **GLP/GEP: yes, unpublished** **...also filed: KCA 8.2.5.3 /01**	**N**	**Y**	**to fulfill data requirement for beta-Cyfluthrin**	**BCS-Irvita**
KIIA 8.5.2/03	**Kimmel, S.**	**2014**	**Beta-cyfluthrin: Effect on the development of sediment-dwelling larvae of Chironomus riparius in water-sediment systems with spiked sediment** **Harlan Laboratories Ltd., Itingen, Switzerland** **BCS-Irvita,** **Report No.: D58731,** **Edition Number: M-481037-01-1** **Date: 2014-03-21** **GLP/GEP: yes, unpublished** **...also filed: KCA 4.1.2 /76** **...also filed: KCA 8.2.5.3 /02**	**N**	**Y**	**to fulfill data requirement for beta-Cyfluthrin**	**BCS-Irvita**
KIIA 8.4/01	Heimbach, F.	1987	Growth inhibition of green algae (Scenedesmus subspicatus) caused by FCR 4545 (techn.) Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/AL 40, Edition Number: M-056512-01-1 Date: 1987-08-27 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
IIA 8.6	**Banman, C. S.; Howerton, J. H.; Lam, C. V.**	**2012**	**Toxicity of Cyfluthrin technical to duckweed (Lemna gibba G3) under static-renewal conditions** **Bayer CropScience LP, Stilwell, KS, USA** **Bayer CropScience,** **Report No.: EBBDL014-1,** **Edition Number: M-437708-02-1** **Date: 2012-09-04** **...Amended: 2012-09-17** **GLP/GEP: yes, unpublished**	**N**	**Y**	**EPA Data Call-In**	**Bayer Crop-Science**
KCA 8.3.1.1 /01	Kleiner, R.	1996	Testing toxicity to honeybee - Apis mellifera L. (laboratory) according to EPPO Guideline No. 170 (1992) - FCR 4545 BioChem GmbH Karlsruhe, Cunnersdorf, Germany Bayer CropScience, Report No.: 96 10 48 079, Edition Number: M-053813-01-1 Date: 1996-11-28 GLP/GEP: yes, unpublished ...also filed: KCA 8.3.1.1.1 /02	N	N		Bayer Crop-Science
KCA 8.3.1.1.1 /01	Davies, L. G.; Carlile, W. R.; Bratby, P.	1985	Report on a laboratory investigation into the toxicity of Cyfluthrin (Baytroid) to honey bees (Apis mellifera) Department of Life Science, Nottingham, United Kingdom Bayer CropScience, Report No.: TOX 1368, Edition Number: M-008790-01-1 Date: 1985-07-30 GLP/GEP: no, unpublished ...also filed: KCA 8.3.1.1 /02	N	N		Bayer Crop-Science

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.8.1.3	Neumann, P.	2001	Acute effects of beta-Cyfluthrin (tech.) on larvae of carabid beetles (Poecilus cupreus) under extended laboratory test conditions Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: NNP/PC006, Edition Number: M-079000-02-1 Date: 2001-10-19 ...Amended: 2002-06-03 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
KIIA 8.9.1/01	Heimbach, F.	1987	Acute toxicity of FCR 4545 (techn.) to earthworms Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: HBF/RG 83, Edition Number: M-053564-01-1 Date: 1987-09-16 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
KIIA 8.9.1/02	**Moser, Th. ; Scheffczyk, A.**	**2009**	**Beta-cyfluthrin FPB-acid: Acute toxicity to the earth-worm Eisenia fetida in an artificial soil test** **ECT Oekotoxikologie GmbH, Floersheim, Germany** **BCS-Irvita,** **Report No.: 09P11RA,** **Edition Number: M-354192-01-1** **Date: 2009-08-21** **GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for soil organisms**	**BCS-Irvita**

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.9.1/03	**Moser, T.**	**2009**	**Beta-cyfluthrin Permethric-acid: Acute toxicity to the earthworm Eisenia fetida in an artificial soil test ECT Oekotoxikologie GmbH, Floersheim, Germany BCS-Irvita, Report No.: 09P10RA, Edition Number: M-356435-01-1 Date: 2009-09-25 GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for soil organisms**	**BCS-Irvita**
KIIA 8.9.2/01	**Kratz, M. A.**	**2013**	**Beta-cyfluthrin-FPB-acid (BCS-AA52287): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil BCS-Irvita, Report No.: kra/Rg-R-143/13, Edition Number: M-468873-01-1 Date: 2013-09-27 GLP/GEP: yes, unpublished**	**N**	**Y**	**new data requirement**	**BCS-Irvita**
KIIA 8.9.2/02	**Kratz, M.**	**2013**	**Beta-cyfluthrin-permethric acid (BCS-AA53389): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil BCS-Irvita, Report No.: kra/Rg-R-157/13, Edition Number: M-468552-01-1 Date: 2013-09-27 GLP/GEP: yes, unpublished**	**N**	**Y**	**new data requirement**	**BCS-Irvita**
KIIA 8.9.2/03	**Pavic, B.**	**2012**	**Effects of beta-Cyfluthrin on reproduction of the predatory mite Hypoaspis aculeifer in artificial soil with 5 percent peat IBACON GmbH, Rossdorf, Germany BCS-Irvita, Report No.: 74501089, Edition Number: M-476271-01-1 Date: 2012-12-17 GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete risk assessment for soil organisms**	**BCS-Irvita**

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.9.2/04	**Frommholz, U.**	**2014**	**Beta-cyfluthrin as: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil BCS-Irvita, Report No.: FRM-Coll-172/14, Edition Number: M-475305-01-1 Date: 2014-01-28 GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete risk assessment for soil organisms**	**BCS-Irvita**
KIIA 8.9.2/05	**Moser, T.; Scheffczyk, A.**	**2005**	**Beta-cyfluthrin FPB-acid: Effects on survival and reproduction of the predaceous mite Hypoaspis aculeifer CANESTRINI (Acari: Laelapidae) in standard soil (LUFA 2.1) ECT Oekotoxikologie GmbH, Floersheim, Germany Bayer CropScience, Report No.: P14HR, Edition Number: M-258697-01-1 Date: 2005-10-12 GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for soil organisms**	**BCS-Irvita**
KIIA 8.9.2/06	**Frommholz, U.**	**2012**	**Beta-cyfluthrin-FPB acid (BCS-AA52287): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil BCS-Irvita, Report No.: FRM-COLL-144/12, Edition Number: M-440962-01-1 Date: 2012-11-05 GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for soil organisms**	**BCS-Irvita**

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.9.2/07	**Moser, T.; Scheffczyk, A.**	**2005**	**Beta-cyfluthrin Permethric-acid: Effects on survival and reproduction of the predaceous mite Hypoaspis aculeifer CANESTRINI (Acari: Laelapidae) in standard soil (LUFA 2.1)** **ECT Oekotoxikologie GmbH, Floersheim, Germany** **Bayer CropScience,** **Report No.: P15HR,** **Edition Number: M-259607-01-1** **Date: 2005-10-27** **GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for soil organisms**	**BCS-Irvita**
KIIA 8.9.2/08	**Frommholz, U.**	**2012**	**Beta-cyfluthrin-permethric acid (BCS-AA53389): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil** **BCS-Irvita,** **Report No.: FRM-COLL-143/12,** **Edition Number: M-440379-01-1** **Date: 2012-10-24** **GLP/GEP: yes, unpublished**	**N**	**Y**	**to complete the risk assessment for soil organisms**	**BCS-Irvita**
KIIA 8.10.1/01	Anderson, J. P. E.	1987	Influence of Cyfluthrin K+L (FCR 4545 techn.) on the microbial mineralisation of carbon in soils Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: AJO/46887, Edition Number: M-054544-01-2 Date: 1987-12-03 GLP/GEP: no, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.10.1/02	Blumenstock, I.	1987	Influence of Cyfluthrin K+L (FCR 4545) on the microbial mineralisation of nitrogen in soils Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: BSI/47987, Edition Number: M-054489-01-2 Date: 1987-12-17 GLP/GEP: no, unpublished	N	N		Bayer Crop-Science
KIIA 8.10.1/03	**Schulz, L.**	**2013**	**Beta-cyfluthrin-FPB acid (BCS-AA52287): Effects on the activity of soil microflora (nitrogen transformation test)** **BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany** **BCS-Irvita,** **Report No.: 13 10 48 016 N,** **Edition Number: M-454537-01-1** **Date: 2013-05-21** **GLP/GEP: yes, unpublished**	**N**	**Y**	**new data re-quirement**	**BCS-Irvita**
KIIA 8.10.1/04	**Schulz, L.**	**2013**	**Beta-cyfluthrin-permethric acid (BCS-AA53389): Effects on the activity of soil microflora (nitrogen transformation test)** **BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany** **BCS-Irvita,** **Report No.: 13 10 48 017 N,** **Edition Number: M-454538-01-1** **Date: 2013-05-22** **GLP/GEP: yes, unpublished**	**N**	**Y**	**new data re-quirement**	**BCS-Irvita**

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KIIA 8.15/01	Caspers, N.; Mueller, G.	1994	Studies on the ecological behaviour of Bulldock Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: 485 A/94, Edition Number: M-053009-01-2 Date: 1994-09-06 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
KIIA 8.15/02	Caspers, N.; Mueller, G.	1994	Studies on the ecological behaviour of Cyfluthrin Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: 478 A/94, Edition Number: M-021811-01-1 Date: 1994-09-06 GLP/GEP: yes, unpublished	N	N		Bayer Crop-Science
	Lambert, M.R.K.	2001	Death from pesticides reviewed among non-target amphibi-ans in sub-saharan africa. Herpetological Bulletin, 78:21-27 published	O	N		LIT
	Bridges, C.M., Semlitsch, R.D.	2000	Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. Cons. Biol. 14, 1490–1499. published	O	N		LIT
	Z. Rao, L. Si, Y. Guan, H. Pan, J. Qiu, G. Li,	2010	Inhibitive effect of cremophor RH40 or tween 80 - based self - microemulsiflying drug delivery system on cytochrome P450 3A enzymes in murine hepatocytes, Journal of Huazhong University of Sc ience and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban, 30 (2010) 562 – 568 published	N	N		LIT

IRV = Irvita Plant Protection, Curacao – a member of Makhteshim Agan Holding B.V., The Netherlands
BCS = Bayer CropScience AG, Monheim, Germany
Grey = Studies were discussed in Volume 3, but were not used in the risk assessment.

Black = Studies used in the risk assessment
Bold = Studies submitted for the first time in support of the renewal approval of beta-Cyfluthrin

B.9.12 References of Guidance documents and open literature:

Annex point / reference number	citation
KII 8.1	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. Available online: www.efsa.europa.eu
KIIA8.1.1/11	Addy-Orduna,L.; Zaccagnini, M-E.; Canavelli, S.B.; Mineau; P. (2011);" Formulated Beta-cyfluthrin Shows Wide Divergence in Toxicity among Bird Species". J. Toxicol., pp. 803451, 10 pp
KII 8.1.4.	Puglis, H.J.; Boon, M.D.(2011); "Effects of Technical-Grade Active Ingredient vs. Commercial Formulation of Seven Pesticides in the Presence or Absence of UV Radiation on Survival of Green Frog Tadpoles".Arch.Environ. Contam. Toxicol., Volume 60, Issue 1, Page 145-155
KII 8.3.1	Liu, W., Gan, J. & Qin, S. (2005). Separation and aquatic toxicity of enantiomers of synthetic pyrethroid insecticides.Chirality, 17:127-133.
KII 8.2.2./ KII 8.3.2	Grace, Nillos Mae, Qin Sujie, Larive Cynthia, Schlenk Daniel, Gan Jay. 2009. "Epimerisation of cypermethrin stereoisomers in alcohols." Journal of agricultural and food chemistry 57 (15): 6938-43. doi:10.1021/jf900921g.
KII 8.2.2./ KII 8.3.2	Perschke, H.; Hussain, M. Chemical isomerisation of deltamethrin in alcohols. J. Agric. Food Chem. 1992, 40, 686–690.
	Lambert, M.R.K. (2001). "Death from pesticides reviewed among non-target amphibians in sub-saharan africa."Herpetological Bulletin, 78:21-27
	Bridges, C.M., Semlitsch, R.D., 2000. Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. Cons. Biol. 14, 1490–1499.
	Z. Rao, L. Si, Y. Guan, H. Pan, J. Qiu, G. Li, Inhibitive effect of cremophor RH40 or tween 80 - based self - microemulsiflying drug delivery system on cytochrome P450 3A enzymes in murine hepatocytes, Journal of Huazhong University of Sc ience and Technology. Medical sciences = Hua zhong ke ji da xue xue bao. Yi xue Ying De wen ban = Huazhong keji daxue xuebao. Yixue Yingdewen ban, 30 (2010) 562 – 568
	Guidance document (GD) for terrestrial ecotoxicology (SANCO/10329/2002)